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Opinion Paper

The Increasing Impact of Laboratory Medicine on Clinical Cardiology

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The practice of cardiology continues to evolve along with a better understanding of the pathophysiology of cardiovascular disease and the development of new therapeutic procedures. Consequently, new demands are being made on the *in vitro* diagnostics industry to improve the performance of existing cardiac markers and to develop novel markers for new cardiac disease indications. Indeed, in the last 20 years there has been a progressive increase in new laboratory tests for markers of cardiac diseases. Several highly sensitive and/or specific assays for the detection of myocardial ischemic damage as well as some immunoassays for cardiac natriuretic hormones, now considered a reliable marker of myocardial function, have become commercially available. In parallel, a growing number of some novel risk factors, which can be assessed and monitored by laboratory methods, have been added to the classical risk factors for cardiovascular disease. Finally, the recent explosion of genetic analysis may soon place at the clinical cardiologist's disposal many laboratory tests for defining the diagnosis at the molecular level, assessing new risk factors, and better targeting the pharmaceutical approaches in patients with cardiovascular disease. In the present article, after a brief description of the analytical tests included in these four groups, each group's impact on clinical cardiology is discussed in detail. Clin Chem Lab Med 2003; 41(7):871–883

Key words: Atrial natriuretic peptide: ANP; Brain natriuretic peptide: BNP; Cardiac disease; Cardiac markers; Cardiac natriuretic peptides; CK-MB; Gene analysis; Troponin.

Abbreviations: ANP, atrial natriuretic peptide; AMI, acute myocardial infarction; BNP, brain natriuretic peptide; CK, creatine kinase; CNH, cardiac natriuretic hormones; CNP, C-type natriuretic peptide; CRP, C-reactive protein; cTnI, cardiac troponin I; cTnT, cardiac troponin T; EF, ejection fraction; ECLIA, electrochemiluminescence "sandwich" immunoassay; ELISA, enzyme-linked immunosorbent assay; IRMA, immunoradiometric assay; LD, lactate dehydrogenase; LDL, low-density lipoprotein; RIA, radioimmunoassay; ROC, receiver-operating characteristic; VCAMs, vascular cell adhesion molecules.

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Introduction

The practice of cardiology continues to evolve along with a better understanding of the pathophysiology of cardiovascular disease and the development of new therapeutic procedures. Since cardiovascular disease is the leading cause of morbidity and mortality in the western countries, the assessment and prevention of cardiovascular risk factors as well as the early diagnosis and management of patients with cardiovascular disease have become primary goals of health care in developed countries.

For these reasons, new demands are being made on the *in vitro* diagnostics industry in order to improve the performance of existing cardiac markers and to develop novel markers for new indications of cardiac disease (1). Indeed, in the last 20 years there has been a progressive increase of new laboratory tests for markers of cardiac diseases, as schematically illustrated in Figure 1. Until about 25 years ago, laboratory medicine placed at clinical cardiology's disposal only a few assays for the detection of cardiac tissue damage (necrosis), such as some enzymatic or electrophoretic methods for creatine kinase (CK) and lactate dehydrogenase (LD), not very specific and sensitive (1, 2). However, in the last 20 years of the 20th century, some highly sensitive and/or specific assays for the detection of myocardial ischemic damage (immunoassays for myoglobin, CK-MB, and cardiac troponin I (cTnI) and cardiac troponin T (cTnT)) (1–7), as well as some immunoassays for cardiac natriuretic hormones (*i.e.*, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and their related pro-peptides), now considered a reliable marker of myocardial function (8–16), have became commercially available (Figure 1).

In parallel, an increasing number of novel risk factors, which can be assessed and monitored by laboratory methods, have been added to the classical risk factors for cardiovascular disease, as recently reviewed (Table 1) (17–48).

Finally, the recent explosion of genetic assay procedures may soon place at the clinical cardiologist's disposal many laboratory tests for defining the diagnosis at the molecular level, assessing new risk factors and better targeting the pharmaceutical approaches in patients with cardiovascular disease, as also recently reviewed in detail (49–65).

Therefore the laboratory tests useful for clinical cardiology can be schematically classified into four groups (Table 2). At present, these groups show a varied impact on clinical cardiology.

Indeed, the introduction of highly specific and sensitive markers of cardiac tissue damage (*i.e.*, the first group of Table 2) into clinical practice has revolution-

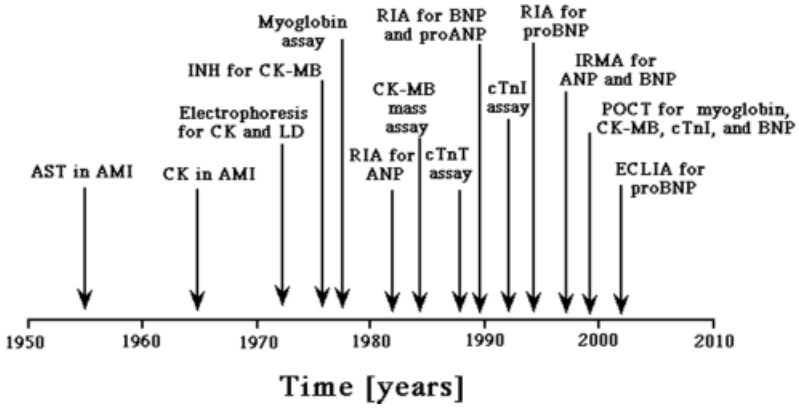

Figure 1 A brief history of assay methods for markers of cardiac tissue damage and myocardial function. AST: aspartate aminotransferase; CK: creatine kinase; LD: lactate dehydrogenase; cTn: cardiac-specific troponin; ECLIA: electrochemiluminescence immunoassay; POCT: point-of-care testing.

Table 1 Classical (well-demonstrated) and some novel (or suggested) cardiovascular risk factors.

Classical risk factors
Age
Sex
Positive family history for cardiovascular disease
Systemic hypertension
Smoking
Dyslipidemia
Physical inactivity
Obesity
Insulin resistance and diabetes
Mental stress and psychosocial factors
Estrogenous status
Novel risk factors
Homocysteine
Fibrinogen
Lipoprotein(a)
Microalbuminuria
γ-Glutamyl transferase (γGT)
Angiotensin II
Uric acid
Markers of coagulation and fibrinolytic function (PAI-1, t-PA, Clot Lysis, d-Dimer and factor V Leiden)
Markers of inflammation (CRP, adhesion molecules (VCAM, ICAM) and pro-inflammatory cytokines (IL-6 and TNFα))
Infectious agents (Cytomegalovirus, Herpes Simplex virus, *Chlamydia pneumoniae*, *Helicobacter pylori*)

Table 2 Schematic classification of laboratory tests for clinical cardiology.

1. Assay for markers of cardiac tissue damage
2. Assay for markers of myocardial function
3. Assay for cardiovascular risk factors
4. Genetic analysis of candidate genes or risk factors for cardiovascular diseases

ized the classification and, at least in part, the pathophysiology of myocardial infarction (66).

The assay for cardiac natriuretic hormones (CNH) (*i.e.*, the second group of Table 2) was recently included as a screening test in the first step of the algorithm for the diagnosis of heart failure by the Task Force of the European Society of Cardiology (16), even if the role of CNH in the identification and management of patients with symptomatic or asymptomatic left ventricular dysfunction remains to be fully clarified (67).

Moreover, the assessment of the lipid profile (by means of the measurement of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides and lipoprotein (a)) (68–70) and, more recently, also C-reactive protein (CRP) (71), have been recommended as laboratory tests for an individual's risk and/or to suggest opportunities for prevention of cardiovascular disease. Other possible cardiovascular risk factors have been suggested (see Table 1) and are now under investigation in order to assess their usefulness, especially in the prevention or follow-up of coronary artery disease.

The genetic analysis of candidate genes or risk factors for cardiovascular diseases (*i.e.*, the fourth group in Table 2) includes several different tests for identifying gene(s) responsible for particular cardiovascular diseases or the analysis of polymorphisms (gene mutations) associated with a particular clinical condition or the increased risk of cardiovascular disease. At present, the impact of these laboratory tests on clinical cardiology is not comparable to that of the other groups reported in Table 2. Indeed, the most common cardiovascular diseases are polygenic disorders, so that more than one candidate gene exists for most of these diseases. Furthermore, conventional molecular diagnostic procedures are usually so time-consuming, expensive and labor-intensive as to discourage the use of these tests in clinical routine (72, 73). Although many new molecular tests concerning possible candidate genes or risk markers for cardiovascular disease have been proposed in the last 20 years, these genetic analyses are currently used in research studies rather than in clinical practice.

The present article will briefly describe the analytical tests included in the fourth group reported in Table 2; each group's impact on clinical cardiology will then be discussed in detail. In order to drastically reduce the number of references, recent reviews, if available, have

been cited instead of many original papers concerning the same topic.

1. Assay for Markers of Cardiac Tissue Damage

The first biochemical marker used in acute myocardial infarction (AMI) was aspartate aminotransferase, which was gradually replaced by creatine kinase (CK) (Figure 1) (2). With the development of electrophoresis methods, CK and lactate dehydrogenase (LD) isoenzymes were introduced as markers with higher specificity than total CK activity. In the last 30 years, several immunoassay methods for cardiac enzymes or structural proteins were set up and introduced into clinical practice, including the assay for the isoenzyme MB of CK (CK-MB), myoglobin, myosin and then troponin I (cTnI) and T (cTnT) (1–4, 68) (Figure 1).

At the same time, the classification, as well as the pathogenesis, of coronary artery disease has progressively changed. Recently, acute coronary syndrome has evolved as a useful operation term referring to any constellation of clinical symptoms that are comparable with acute myocardial ischemia (2, 66, 74) (Figure 2). These changes can be at least partly attributed to the introduction in clinical practice of highly sensitive and specific immunoassay methods for cardiac tissue damage, such as cTnI or cTnT and, in part, CK-MB mass.

The advent of sensitive and specific biomarkers necessitated re-evaluation of established definitions of myocardial infarction (66). If we accept the concept that any amount of myocardial necrosis caused by ischemia should be labeled as an infarct, then an individual who was formerly diagnosed as having severe, stable or unstable angina pectoris might be diagnosed today as having had a small myocardial infarction (66).

Indeed, the ACC/AHA (American College of Cardiology/American Heart Association) 2002 updated guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction (74) recommend that biomarkers of cardiac injury should always be measured in these patients for early risk stratification. In this clinical setting, patients with unstable angina can be distinguished from those with non-ST-segment elevation myocardial infarction only by means of assays for specific cardiac damage markers (*i.e.*, cTnI or cTnT) (74) (Figure 2).

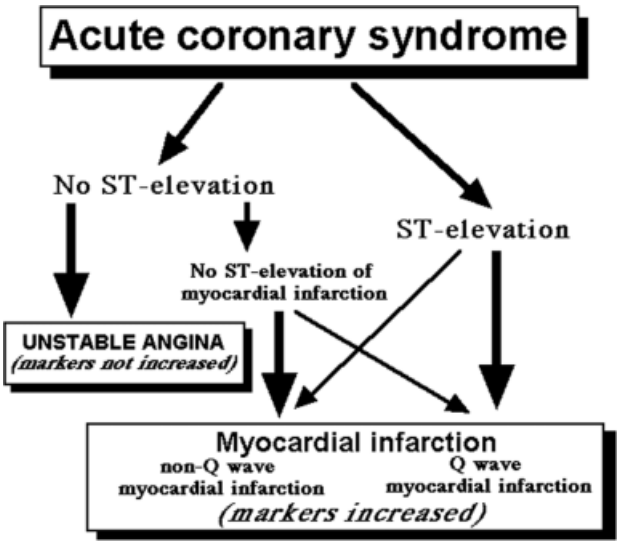

Figure 2 Clinical classification of acute coronary syndrome (modified from reference 74).

Table 3 Some clinical conditions in which the circulating levels of troponin are increased, without overt artery coronary disease (modified from references 83, 84).

Myocarditis/pericarditis
Congestive heart failure
Systemic arterial hypertension
Systemic arterial hypotension (especially if associated with cardiac arrythmias)
Critically ill patients
Hypothyroidism
Cardiac trauma
Myocardial toxicity from cancer therapy
Pulmonary embolism
Episode rejection of a cardiac transplant
Postoperative non-cardiac surgery
Chronic renal failure
Amyloidosis
Sepsis

The introduction of techniques for measuring cardiac troponin allows for very sensitive and very specific detection of minimal (microscopic) quantities of myocardial necrosis (66). In this setting, two important clinical points should be taken into account. First, it is important to underline that increased levels of a marker reflect myocardial damage but do not indicate its mechanism (4). Thus, an increased value, in the absence of clinical evidence of ischemia, should prompt a search for other causes of cardiac damage (Table 3) (66). Moreover, currently available analyses demonstrate no threshold below which elevations of troponins are harmless and without negative implications for prognosis; therefore the assay for cardiac damage is essential for the assessment for risk stratification and prognosis of patients with acute coronary syndrome (3, 5, 66, 68, 74–80).

Cardiac-specific troponin is the preferred marker, and if available, it should be measured in all patients with typical chest pain; however, CK-MB by mass assay is also acceptable (5, 66, 68, 74). Moreover, in patients with negative cardiac markers within 6 hours of the onset of pain, another sample should be drawn in the 6- to 12-hour time frame. For patients who present very early in the Emergency Department, an early marker of cardiac injury, such as myoglobin or CK-MB subforms, should be considered in addition to a cardiac troponin (74, 75). Measurement of total CK, glutamic-oxaloacetic transaminase, LD and LD isoenzymes should not be used to diagnose cardiac damage (66).

Since the assay for cardiac damage by means of a sensitive and specific biomarker serves as the cornerstone of the new definition of myocardial infarction (66), the laboratory now assumes a pivotal role in the

diagnosis and follow-up of patients with acute coronary syndrome. However, there are some important technical and methodological implications in this increasing role of the laboratory in this clinical setting.

As suggested by the consensus document of the joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction (66), an increased value for a cardiac marker (such as troponin) should be defined as a measurement exceeding the 99th percentile of a reference control group. Moreover, acceptable imprecision at the 99th percentile for each assay should be defined as ≤ 10%. Although it is recommended that each individual laboratory should confirm the range of reference values in their specific setting (66), assay standardization would undoubtedly be preferable (81–85).

Unfortunately, unlike cTnT, which is manufactured by only one *in vitro* diagnostic company and therefore has *de facto* standardization, numerous manufacturers have developed their own cTnI assays, leading to a situation in which cTnI measurements using different methods on identical specimens have been shown to differ by more than 100-fold (81). Furthermore, many (or even most) of the commercially available assay methods for troponin do not actually show the requested imprecision and/or report the 99th percentile value of the reference control group (82–84). Therefore, a standardization of troponin assays is mandatory in order to achieve common reference and decision limits (83).

Finally, the indiscriminate assay for troponin in all patients with chest pain may lead to an increase in "false positives", since troponin levels can be increased in clinical conditions other than acute coronary syndrome (Table 3) (84, 85). However, as recently suggested (84), these clinical conditions may be becoming an interesting area for further development in which the troponin assay is clinically useful in order to study situations other than ischemic heart disease, where a subtle degree of myocardial tissue injury can be present. It is also important to underline that some of the clinical conditions reported in Table 3 are very frequent in clinical practice (such as severe heart failure, sepsis, high-dose cardiotoxic chemotherapy, pulmonary embolism or critically ill patients) and that a significant relationship has been often observed between cardiac troponin values and disease severity (86).

2. Assay for Markers of Myocardial Function

The presence of granules in some heart atria cells of guinea pigs were first reported by Kirsh in 1956 (87); similar findings were then confirmed in the atria of all mammalian species, including man (88). The shape, structure and position (in the midst of an extremely well-developed Golgi complex) of these granules are more similar to those of secretory granules found in peptide-secreting hormone cells than those of other cell organelles (such as lysosomes). After the demonstration by Marie *et al.* (89) that the granularity of cardiocytes can be affected by sodium and water balance, de Bold *et al.* (90) made the crucial observation that in rats, extracts of atrial homogenates, when administered intravenously to the animals, resulted in a rapid, massive and short-lasting diuresis and natriuresis.

This key observation has been the origin of an explosion of studies concerning the isolation, purification and identification of a family of peptides (*i.e.*, CNHs) localized in the granules and responsible for the natriuretic effects. These studies demonstrated that not only the atria but also the ventricles are able to synthesize and secrete the CNH, especially in congestive heart failure (8–15).

It was therefore well-established that the heart has an endocrine function since it synthesizes and secretes a family of peptide hormones with potent diuretic, natriuretic, vascular smooth muscle-relaxing activity as well as complex interactions with the hormonal and nervous systems (8–15). Taking these findings into account, the heart now appears to be a complex organ, with both a mechanical and a neuroendocrine function.

In the clinical practice, the mechanical function (*i.e.*, pumping the blood throughout the body) has been assessed with the classical invasive and non-invasive cardiological investigations (such as electrocardiogram, echocardiography, nuclear medicine scintigraphy or cardiac catheterization and hemodynamic studies).

The neuroendocrine function of the heart includes not only the CNH system but also the sympathetic and parasympathetic systems, the endothelin system and the renin-angiotensin system, as well as other factors produced in cardiac tissue (91). The functional activity of these neuro-hormone systems are assessed by means of specific tests, which measure the levels of these factors in blood or cardiac tissue as well as their cardiac expression, receptor binding and *in vivo* metabolism (92).

It is important to underline that mechanical and neuroendocrine functions both contribute to the overall cardiac function, and, although separate, are inter-dependent functions, mutually affected by many and complex feedback mechanisms. A corollary of this assumption is that the assay of the neuroendocrine system and clinical investigations of cardiac pump function offer different, but complementary, information about cardiac function. Therefore, both mechanical and neuroendocrine functions should always be separately tested by suitable methods in order to achieve a more complete knowledge of the role played by the heart in all the physiological and clinical conditions.

From this point of view, laboratory medicine should now assume a greater importance in the clinical assessment of cardiac function as well (16). Although the reliable role of CNH in identification and management of patients with symptomatic or asymptomatic ventricular dysfunction remains to be fully clarified (67), the clinical usefulness of CNH (especially BNP or N-terminal proBNP) assay in screening for heart disease (93), in stratification of patients with congestive heart failure (94), in detecting left ventricular systolic and/or diastolic dysfunction (95) and in differential diagnosis of dyspnea (96, 97), has been confirmed even more recently.

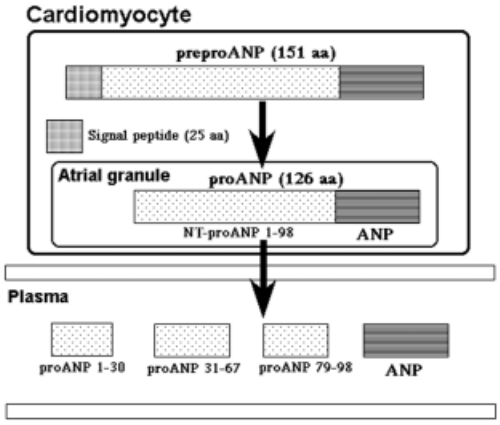

Figure 3 Synthesis and secretion of ANP. Atrial natriuretic peptides are a family of peptides derived from a common precursor, called preproANP, which in humans contains 151 amino acids and has a signal peptide sequence containing 25 amino acids at its amino-terminal end. The pro-hormone is stored in the atrial granule as a 126-amino acid peptide, $proANP_{1-126}$, which is produced by cleavage of the signal peptide. When appropriate signals for hormone release are given, $proANP_{1-126}$ is further split into an N-terminal fragment, $proANP_{1-98}$, and the C-terminal peptide $proANP_{99-126}$ (generally called ANP), which is considered to be the biologically active hormone. Moreover, N-terminal $proANP_{1-98}$ can be degraded *in vivo* with the production of at least three different shorter peptides (*i.e.*, N-terminal $proANP_{1-30}$, $proANP_{31-67}$ and $proANP_{79-98}$), some of these may be biologically active (9).

CNHs are a complex family of related peptides with similar peptide chains as well as degradation pathways. CNHs include atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), while other natriuretic peptides, such as C-type natriuretic peptide (CNP) and urodilatin, structurally related to the ANP/BNP peptide family, are not produced and secreted by cardiac tissues but by other tissues (8–15). CNHs are derived from common precursors, prepro-hormones (*i.e.*, preproANP and preproBNP), which contain a signal peptide sequence at the amino-terminal end (Figures 3 and 4). The pro-hormones (proANP and proBNP) are produced by cleavage of the signal peptide; then the pro-hormone peptide is further split into a longer NH_2-terminal fragment (N-terminal proANP or N-terminal proBNP), and a shorter COOH-terminal peptide (ANP or BNP), which are secreted into the blood in equimolar amounts. However, ANP and BNP have shorter plasma half-lives compared to N-terminal propeptides (proANP and proBNP) and consequently they also have lower plasma concentrations (Table 4) (8–15).

Studies of structure-activity relationships have shown the importance of the central ring structure of CNHs formed by a disulfide bridge between the two cysteine residues (*i.e.*, this cysteine bridge is necessary for the binding to the specific receptors). Disruption of this ring by hydrolytic cleavage leads to loss of biological activity. For this reason, only ANP and BNP, which present the disulfide bridge in the peptide chain, share the typical hormonal activity of CNHs, while the N-terminal proANP and proBNP do not.

The use of CNHs assay as a screening method in patients with heart failure implies that reliable assays for CNHs must be available for all laboratories. CNHs and

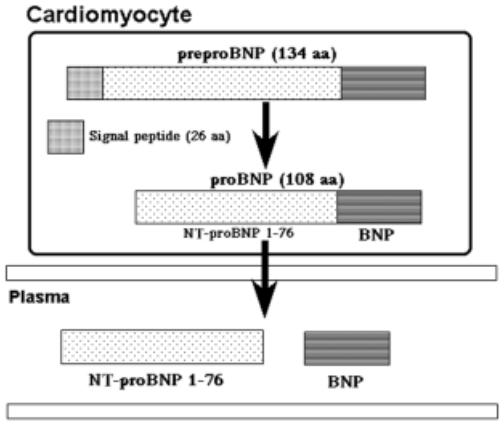

Figure 4 Synthesis and secretion of BNP. BNP derives from a precursor, called preproBNP, which in humans contains 134 amino acids and has a signal peptide sequence at its amino-terminal end containing 26 amino acids. The pro-hormone containing 108 amino acids ($proBNP_{1-108}$) is produced by cleavage of the signal peptide, when appropriate signals for hormone release are given. $ProBNP_{1-108}$ is further split into an N-terminal fragment, $proBNP_{1-76}$, and the C-terminal peptide $proBNP_{77-108}$ (generally called BNP), which is considered to be the biologically active hormone. BNP is preferentially produced and secreted in the ventriculum without storage in the granules, although some secretory granules can contain this hormone. For this reason, in contrast to ANP, regulation of BNP synthesis and secretion occurs mainly at the level of gene expression.

Table 4 Mean analytical sensitivity, normal values (mean ± SD) and range (minimum and maximum values) of some commercial competitive (EIA) and non-competitive (IRMA, ELISA and ECLIA) immunoassays for CNH measured in the author's laboratory.

Method	Sensitivity (pmol/l)	Normal values (pmol/l)	Range (pmol/l)
IRMA ANP	0.73	5.6±3.6	0.2–16.6
IRMA BNP	0.75	2.9±2.7	0.1–12.4
ELISA proANP	76.9	731±628	43–1502
IRMA proANP	40.5	228±99	63–422
EIA $proANP_{1-30}$	9.5	708±251	44–1289
EIA $proANP_{31-67}$	38.4	1422±790	193–3339
EIA Nt-proBNP	13.6	246.8±120.1	64–488
EIA Mid-proBNP	4.0	117.5±100.3	0.2–368
ECLIA NT-proBNP	0.6	6.1±4.1	1.7–21.1

IRMA ANP (Shionogi & Co., Ltd, Osaka, Japan); IRMA BNP (Shionogi & Co.); ELISA proANP (Biomedica Gruppe, Vienna, Austria); IRMA proANP (Shionogi & Co.); EIA proANP 1–30 (Biomedica Gruppe); EIA proANP 31–67 (Biomedica Gruppe); EIA NT-pro BNP (code BI-20852, Biomedica Gruppe), which uses an antiserum against the N-terminal $proBNP_{8-29}$ peptide fragment; EIA Mid-proBNP (code BI-20862, Biomedica Gruppe), which uses an antiserum against the N-terminal $proBNP_{32-57}$ peptide fragment; ECLIA NT-proBNP (proBNP Elecsys System 2010, Roche Diagnostics S.p.A., Monza, Italy).

related peptides were measured for many years with competitive (RIA) or non-competitive (IRMA) immunoassay methods using radioactive labels (98). The main advantage of immunoassays using radioactive labels, compared to other immunoassays, is the lower cost. However, radioactive tracers are less stable and safe than non-radioactive labels and can only be used in a few clinical laboratories (98). Furthermore, these assays suffer from poor sensitivity and/or specificity and are also time-consuming and labor-intensive, so that a new generation of assay methods for CNH, as well as a standardization of these methods, are now necessary (98).

Three main problems should be addressed in order to allow a widespread propagation of CNH assays in clinical practice, including their use in emergency and primary care. First, increased analytical sensitivity should be achieved. This improvement should allow the determination of all the normal range of CNHs with an acceptable imprecision (CV $\leq$ 10%); this goal is particularly pressing for BNP assay. Second, an increase in analytical specificity (accuracy), and/or a standardization, is also necessary, especially for the assay of proANP and proBNP related peptides (see for example the great variations among proANP and proBNP values in normal subjects, obtained with different immunoassay methods, as reported in Table 4). Third, an increase in practicability is also necessary. Indeed, it is not possible to spread the routine measurement of CNH throughout the clinical practice, including emergency and primary care, without a new generation of immunoassay methods, permitting the determination of CNH in a few minutes.

A fully automated fluorescent immunoassay for BNP assay, dedicated to point-of-care testing, has been recently tested in several studies including patients with heart failure (13, 95, 96). This method allows the rapid assay (one assay is performed within 30 min) of either one fresh whole blood or plasma sample at a time and, considering their characteristics, is better indicated for the BNP assay in ambulatory and coronary or emergency units, where usually only a few (preferentially whole-blood) samples are measured in a short time (99).

More recently, a fully automated electrochemiluminescence "sandwich" immunoassay (ECLIA) for NT-proBNP has also been developed (100). The analytical characteristics of this ECLIA method seem to fit well with those requested by a second generation assay for CNH (100). In particular, using a dedicated automated analyzer it is possible to perform an NT-proBNP assay within 30 min. Moreover, the reproducibility of the ECLIA method is much better than that of RIA or IRMA methods (100). Furthermore, new generation methods for BNP should soon become commercially available (101, 102).

There are not only methodological problems for laboratory medicine to solve in accepting the challenge of proposing biological markers for cardiac function; some problems also exist in the clinical interpretations of CNH assay results.

Table 5 Some common diseases in which plasma cardiac natriuretic peptides have been found to be altered, compared to healthy subjects.

Diseases	Hormone levels
A) Cardiac diseases	
Heart failure	Greatly increased
AMI (first 2–3 days)	Greatly increased
Essential hypertension with CMP	Increased
B) Pulmonary diseases	
Acute dyspnea	Increased
Obstructive pulmonary disease	Increased
C) Endocrine and metabolic diseases	
Hyperthyroidism	Increased
Hypothyroidism	Decreased
Cushing's syndrome	Increased
Primary aldosteronism	Increased
Addison's disease	Normal or increased
Diabetes mellitus	Normal or increased
D) Liver cirrhosis with ascites	Increased
E) Renal failure (acute or chronic)	Greatly increased
F) Paraneoplastic syndrome	Normal or increased
G) Subarachnoid hemorrhage	Increased

AMI = acute myocardial infarction; CMP = cardiomyopathy with left ventricular hypertrophy.

First, circulating levels of CNHs (especially those of BNP and N-terminal proBNP) are greatly affected by age and gender, so that age- and gender-related cut-off values should be taken into account in clinical practice (103–105). Second, several physiological (such as physical activity and pregnancy) or clinical conditions (including some cardiovascular, endocrinological, renal, liver and neoplastic diseases) can affect the circulating levels of CNHs (9) (Table 5). Third, certain drugs can also affect the CNH system; in particular, digitalis, β-blocker agents, ACE inhibitors and diuretics (9), which are routinely administered to patients with cardiovascular diseases.

Furthermore, all the above-mentioned factors can affect the cardiac neuroendocrine system and the mechanical pump function in different ways and with different (even contrasting) effects. For these reasons, it is theoretically conceivable that results of echocardiographic (or hemodynamic) investigations could not be strictly related to those of neuro-hormone assays in some physiological or clinical settings. Indeed, it is well known that the left ventricular ejection fraction (EF) and logarithmic transformation of plasma BNP values are strictly related in patients with different degrees of heart failure, but EF explains only a part of the variation of BNP values (for example, only 43.6% for the regression reported in Figure 5). As also demonstrated by data reported in Figure 5, some patients with heart failure can have normal BNP values but decreased EF values (or *vice versa*), so a total of 21% of patients with heart failure are classified differently by the two tests. Evidently, the number of patients classified differently strongly depends on the cut-off values chosen for EF and logBNP, respectively. Furthermore, it is conceiv-

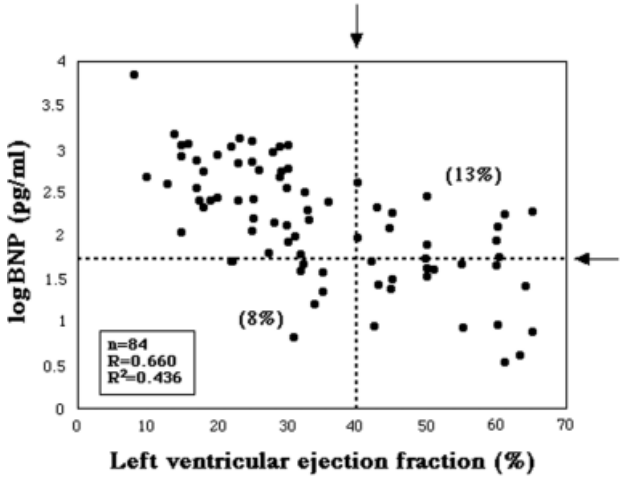

Figure 5 Linear regression between BNP and left ventricular ejection fraction (EF) values (assessed by echocardiography) in patients with cardiac disease and different degrees of heart failure. Cut-off values of "normal" population for both logBNP and EF are also indicated by arrows.

able that the regression between BNP and EF values should be less close if a community population is tested, since some subjects can present some pathophysiological conditions or assume some drugs, which can differently affect the cardiac neuroendocrine and pump functions.

The comparison of two recent studies (106, 107), concerning the evaluation of the CNH assay as a screening method in a general population, could aid in explaining certain problems in the interpretations of CNH assay results and especially in their comparison with echocardiographic or other clinical results.

The first study analyzed the Framingham Heart Study cohort (3177 patients) using BNP and N-terminal proANP in the evaluation of left ventricular hypertrophy and systolic dysfunction in a community population (106). The presence (or absence) of the disease (*i.e.*, left ventricular hypertrophy and/or systolic dysfunction) was evaluated by using mainly (if not only) the echocardiographic results. The area under curve (AUC) of receiver-operating characteristic (ROC) analysis of CNH assay for identifying echocardiographic abnormality was about 0.75. In particular, gender-related BNP cut-off values ranging from 45 to 50 pg/ml (approx. 13 to 14.4 pmol/l) yielded good specificity (*i.e.*, 0.95) for detecting both left ventricular systolic dysfunction and elevated left ventricular mass, but a very poor sensitivity (*i.e.*, 0.27 in men and 0.13 in women) for detecting elevated left ventricular mass (106).

The aim of the second study was to examine the validity of plasma BNP measurement (with the same IRMA method as the other study) for detection of various cardiac abnormalities in a rural Japanese population (1098 subjects) with a low prevalence of coronary heart disease and left ventricular systolic dysfunction (107). The diagnosis was carried out by two independent cardiologists based on each subject's medical questionnaire, chest radiograph, ECG and echocardiographic report. The optimal threshold for identification was a BNP concentration of 50 pg/ml (14.4 pmol/l) with a sensitivity of 89.7% and specificity of 95.7%. The area under the ROC curve was 0.970. The positive and negative predictive values at the cut-off level were 44.3% and 99.6%, respectively.

The conclusions of these two studies, though similar in aims as well as in clinical and experimental protocols, were strongly conflicting. The Japanese study suggested that measurement of plasma BNP concentration is a very efficient and cost-effective mass screening technique for identifying patients with various cardiac abnormalities regardless of etiology and degree of left ventricular systolic dysfunction (107), while the Framingham study suggested only limited usefulness of CNH assay as a mass screening tool for this clinical condition (106). Although it is conceivable that these discrepancies are mainly due to the different populations enrolled and to the different inclusion and exclusion criteria chosen in these two studies, these conflicting results may be explained at least in part by the different "gold standards" used for the comparison with the CNH assay results. In the Japanese study the subjects were divided into "affected" or "not-affected" subjects on the basis of a diagnosis made by two independent cardiologists using all the clinical information available, while in the Framingham study the individuals were stratified according to the echocardiographic report.

Even if it is conceivable that CNH assay may have only a limited usefulness as a screening method for heart failure in a general population, the use of only the echocardiographic report for the stratification of individuals (or patients) for comparison with CNH assay may be misleading since these two tests have different pathophysiological relevance and provide different clinical information.

The use of CNH assay as a biomarker of cardiac function in clinical practice may require a reassessment of the paradigms currently used in clinical cardiology to evaluate the presence and severity of heart failure. According to this point of view, patients with heart failure should be stratified following the evaluation of ventricular function by means of classical invasive and non-invasive cardiological investigations together with the results of CNH assay as well as clinical symptoms.

3. Assay for Cardiovascular Risk Factors

The measurement of many "classical" or "novel" risk factors has been suggested in order to evaluate an individual's risk of atherosclerosis in general and/or in particular for coronary artery disease; only some of all these risk factors, probably the more interesting or frequently cited, have been reported in Table 1 (18–24, 28, 33, 35, 46, 68–71). However, at the present time, only the assessment of lipid profile and, more recently, the measurement of CRP, have been recommended as routine laboratory tests for the evaluation of the individual's risk and/or prevention of cardiovascular disease (69–71).

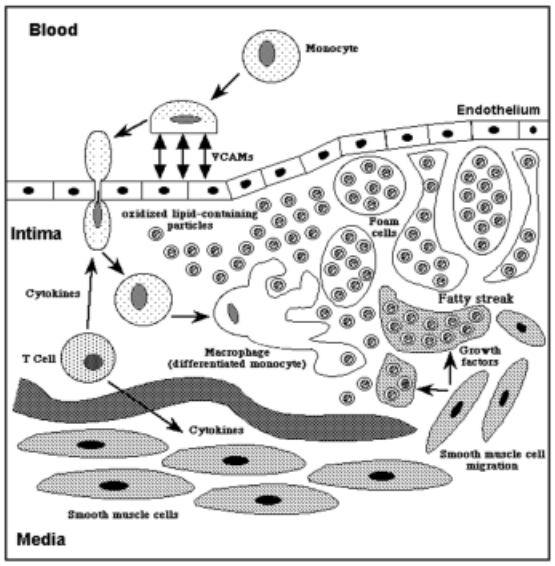

Figure 6 Schematic representation of an atherosclerotic plaque formation.

Contrary to the first groups of laboratory tests for clinical cardiology reported in Table 2, this third group includes some assays that are used mainly for primary or secondary prevention of coronary artery disease rather than for the diagnosis or severity evaluation of cardiac disease (30, 69).

Due to the great number of substances suggested (Table 1), it is clearly impossible to discuss the clinical importance of all these factors in detail. However, it could be interesting to underline some common pathophysiological characteristics among all these substances indicated as candidate risk factors for coronary artery disease. Generally speaking, nearly all these factors are strictly related to the pathophysiological process of atherosclerosis plaque formation and/or evolution (Figure 6).

Atherosclerosis starts with localized injury to the endothelium causing endothelial dysfunction; this particularly occurs at sites of high shear stress (107, 108). Dysfunctioning (activated) endothelial cells express vascular cell adhesion molecules (VCAMs), which can also be measured in the blood, and, theoretically, the assays of these molecules could be used to monitor disease progression or to detect sub-clinical lesions (108).

Monocytes bind to the VCAMs and then migrate into the sub-endothelial region. LDLs also enter the vessel wall and then are oxidized or chemically modified by several mechanisms (such as glycation) (107). Some of these mechanisms need the activation and recruitment of leukocytes, including monocyte and T lymphocytes. Moreover, polymorphonuclear leukocytes are preferentially recruited by E- (endothelial) or P- (platelet) selectins, which can be present in early atheroma plaques, especially when bacterial pathogens (or other inflammatory agents) are also present in the lesion (107–110). The current concept of directed migration of leukocytes involves the action of chemo-attractant cytokines (such as MCP-1, IP-10, I–TAC and MIG) (107, 108, 110).

The monocyte, once recruited to the arterial intima, can there imbibe lipids and become a "foam cell". Once macrophages have taken up residence in the intima and become foam cells, they not infrequently replicate due to the action of some factor (such as macrophage colony-stimulating factor, interleukin-3 and granulocyte-macrophage colony-stimulating factor) (107, 108). T cells elaborate inflammatory cytokines such as γ-interferon and tumor necrosis factor (TNF)-β, which in turn can stimulate macrophages as well as vascular endothelial cells and smooth muscle cells. As this inflammatory process continues, the activated leukocytes and intrinsic arterial cells can release fibrogenic mediators, including a variety of peptide growth factors, which can promote replication of smooth muscle cells and contribute to elaboration by these cells of a dense extracellular matrix characteristic of more advanced atherosclerosis lesions (109, 110).

Whereas the early events in atheroma initiation involve mainly endothelial dysfunction and recruitment and accumulation of leukocytes, the subsequent evolution of atheroma into more complex plaques involves smooth muscle cells, which can accumulate and multiply by cell division into the intima, attracted by some factors (such as platelet-derived growth factor; PDGF) (106).

The extracellular matrix makes up much of the volume of an advanced atherosclerotic plaque. The biosynthesis of extracellular matrix molecules counters breakdown, catalyzed in part by catabolic enzymes known as matrix metalloproteinases (MMPs), which can also be considered as a biomarkers for the evolution of atheroma plaques (111–115).

The smooth muscle cell is not alone in its proliferation and migration; endothelial migration and replication also occur with the action of some angiogenesis factors (such as fibroblastic and vascular endothelial growth factors, and oncostatin M) as plaques develop in microcirculation, characterized by plexuses of newly formed vessels (116). Finally, plaques often develop areas of calcification and may also contain proteins specialized in sequestering calcium and thus promoting mineralization (107).

Many of the above-mentioned cytokines (as well as adhesion and matrix molecules and other pro- and anti-inflammatory factors) play an important role in some disease states, including the formation of atheroma. Clinicians hope one day to induce remission of cytokine-mediated pathologic lesions by manipulating *in vivo* levels and proportions of pro- and anti-inflammatory cytokines (108). This implies that reliable assay methods for circulating and/or tissue levels of cytokines, adhesion and matrix molecules, and other pro- and anti-inflammatory factors, must be available in order to monitor the progression or remission of the disease. At present, these substances are measured in tissue with immunochemistry or molecular biology methods, and in biological fluids mainly with ELISA methods (108); however, all these are "research", time-

consuming and labor-intensive methods, and consequently unsuitable for clinical practice. Therefore the set-up of new immunoassay methods, which are commercially available and show better analytical characteristics (fully automated and low-cost) and performance (better imprecision, accuracy and sensitivity) is mandatory for their clinical use.

Reactive oxygen species (such as superoxide anion, hydrogen peroxide, peroxynitrite and hydroxyl radical) seem to play a pivotal role in the pathogenesis of coronary artery disease, and especially in ischemia-reperfusion injury (117). Free radicals exert their cytotoxic effects by producing several metabolic dysfunctions, including peroxidation of polyunsaturated fatty acids in membrane or plasma lipoproteins, direct inhibition of mitochondrial respiratory chain proteins, inactivation of membrane sodium channel, chemical modification of other proteins, and even DNA damage (117). All these modified substances (such as damaged DNA and oxidized LDL) can be measured in patients with coronary artery disease and can be theoretically used for monitoring the atherosclerotic process and/or for evaluating tissue damage, and even for designing new paradigms of therapy (117–121).

Endothelial activation may contribute to thrombosis *in situ* in atherosclerosis; many inflammatory mediators found in atherosclerotic plaques can augment the tissue factor, a procoagulant molecule that greatly multiplies the activity of coagulation factors VIIa and Xa (122). Coronary thrombosis is also an important determinant of prognosis in patients with acute coronary syndromes (5, 123, 124). The measurement of markers of activation of the coagulation/fibrinolytic system with laboratory methods (125) could be important for risk stratification of patients with acute coronary syndromes since increased peptide activation may reflect a tendency toward the progression of coronary thrombosis (5). However, large-scale trials of fibrinolytic activity in unstable coronary artery disease are still lacking and, therefore, the importance of such measurement for assessing prognosis remains unsettled (5).

4. Genetic Analysis of Candidate Genes or Risk Factors for Cardiovascular Diseases

The recent explosion of genetic analysis may soon place at the clinical cardiologist's disposal many new laboratory tests for defining the diagnosis at the molecular level, assessing new risk factors and better targeting the pharmaceutical approaches in patients with cardiovascular diseases (52, 60, 126).

The genetic analysis of candidate genes or risk factors for cardiovascular diseases includes several and disparate tests concerning the identification of gene(s) responsible for particular cardiovascular diseases or the analysis of polymorphisms (gene mutations) associated with a particular clinical condition or with an increased risk for cardiovascular disease.

Individual genomes are 99.9% identical, with only 0.1% of the genoma showing polymorphisms (60, 126). However, human disease genes show enormous variation in their allelic spectra; that is, in the number and population frequency of the disease-predisposing alleles at the loci (127). For some genes, there are a few predominant disease alleles; for others, there is a wide range of disease alleles, each relatively rare. The allelic spectrum is important since disease genes with only a few deleterious alleles can be more readily identified and are more amenable to clinical testing (127). Unfortunately, little is known about the allelic spectrum for genes underlying common disorders, such as coronary artery disease.

At the present time, the impact on clinical cardiology of molecular laboratory tests is not comparable to that of the other groups reported in Table 2. Indeed, the most common cardiovascular diseases are polygenic disorders, so that there is more than one candidate gene for the major part of these diseases. For example, atherosclerotic diseases, including coronary artery disease, result from a dynamic, lifelong interaction between genetic, environmental and behavioral factors (60, 126, 128). Classic risk factors for myocardial infarction, such as hypertension, diabetes and dyslipidemia, result from multiple susceptibility loci interacting with behaviour and the environment. This represents a difficult challenge for clinical medicine in order to attempt to associate genes with physiological functions and mutations with disease (60).

On the other hand, a smaller number of cardiac diseases could be due to chromosomal or single-gene disorders. Malformation of the heart and blood vessels account for the largest number of human birth defects, occurring in about 1% of all live births, and some of these can be due to chromosomal or single-gene disorders (129, 130). Moreover, some of the idiopathic dilated and hypertrophic cardiomyopathies, especially familial forms, could be also due to single-gene, heritable genetic defects (49, 50, 54, 61, 64). Finally, even some forms of cardiac arrhythmias can be inherited (55, 56, 65). Clearly, genetic molecular testing, when available, could confirm the clinical diagnosis, provide the opportunity for appropriate genetic counselling, and indicate new paradigms in the treatment for all these clinical conditions.

Even if a growing number of molecular tests, concerning possible candidate genes or risk markers for cardiovascular disease, have been proposed in the last 20 years (49–57, 60, 126), these genetic analyses are currently used only in research studies rather than in clinical practice. Indeed, conventional molecular diagnostics are usually so time-consuming, expensive, and labor-intensive as to dissuade from using these tests in clinical laboratory medicine at present (71, 72).

In only a few short years, hybridization array technology, which enables the performance of thousands of simultaneous hybridization reactions on a solid substrate within a single analytical procedure, has evolved from a theoretical construct to practical reality (72). This technical approach should offer previously unimaginable opportunities for diagnostic application,

ranging from gene sequencing and detection of genetic polymorphisms to measurement of gene expression profiles of human tissues, including heart tissue.

Rapid progress in this field over the last few years has been driven by a unique technological convergence of microfabrication, robotics and bioinformatics; however, these new molecular tests are unlikely to replace traditional testing in the immediate future (71, 72). Indeed, the cost and complexity of this technology tends to restrict its initial applications to special diagnostic conditions, where the information obtained cannot be provided by any other method. Therefore, an increased automation is necessary with a parallel reduction of the level of technical expertise required to perform the tests, as well as of cost, and also a better integration with other laboratory instrumentation, before welcoming these new molecular technologies into the mainstream of laboratory testing.

Conclusions

Richard J. Bing in his preface of the book "*Cardiology – The Evolution of the Science and the Art*" writes that he "has lived though three phases of cardiology: a purely clinical phase, where bedside diagnosis went hand in hand with therapeutic nihilism; a period that stressed the dynamic of circulation, where some cardiac diseases were explained on a purely hemodynamic basis; and, finally, a time when invasive procedures together with biochemistry, biophysics, and molecular biology became the primary influence" (132).

The increasing impact of laboratory medicine on clinical cardiology can be well reflected in these words. Until about 25 years ago laboratory medicine placed at the clinical cardiologist's disposal only a few and unspecific assays; however, recently a rapidly growing number of classical clinical chemistry procedures and immunoassay methods and, more recently, genetic/molecular tests, have been suggested to be useful for the clinical diagnosis of cardiovascular diseases and/or for the follow-up of affected patients. Some of these diagnostic tests (such as troponin assay) have already significantly contributed to changing some pathophysiological and/or clinical paradigms, while the role of other tests (which represent the major part) in the identification and management of patients with symptomatic or asymptomatic cardiovascular diseases remains to be fully clarified.

The future challenge shows two fronts: on the one hand, the *in vitro* diagnostic industry should invest more in novel or more reliable tests dedicated to clinical cardiology; on the other hand, "evidence-based medicine" studies should indicate the best tests for clinical practice. In the middle, the clinical laboratories should select and evaluate the candidate methods objectively, with special regard for the problem of standardization.

References

1. Wu AHB, McCord RG. New biochemical markers for heart disease. In: Wu AHB, editor. Cardiac markers. Totowa: Humana Press 1998:281–94.
2. Wu AHB. Introduction to coronary artery disease (CAD) and biochemical markers. In: Wu AHB, editor. Cardiac markers. Totowa: Humana Press 1998:3–20.
3. Penttila I, Penttila K, Rantanen T. Laboratory diagnosis of patients with acute chest pain. Clin Chem Lab Med 2000; 28:187–97.
4. Plebani M. Biochemical markers of cardiac damage: from efficiency to effectiveness. Clin Chim Acta 2001; 311:3–7.
5. Galvani M, Ferrini D, Ghezzi F, Ottani F. Cardiac markers and risk stratification: an integrated approach. Clin Chim Acta 2001; 311:9–17.
6. Blomkalns AL, Gibler WB. Markers and the initial triage and treatment of patients with chest pain. Cariovasc Toxicol 2001; 1:111–5.
7. Shebuski RJ. Utility of point-of-care diagnostic testing in patients with chest pain and suspected acute myocardial infarction. Cur Opin Pharmacol 2002; 2:160–4.
8. Sagnella GA. Measurement and significance of circulating natriuretic peptides in cardiovascular diseases. Clin Sci 1998; 95:519–29.
9. Clerico A, Iervasi G, Mariani G. Clinical relevance of the measurement of cardiac natriuretic peptide hormones in humans. Horm Metab Res 1999; 31:487–98.
10. Boomsma F, Van den Meiracker AH. Plasma A- and B-type natriuretic peptides: physiology, methodology and clinical use. Cardiovasc Res 2001; 51:442–9.
11. Suzuki T, Yamazaki T, Yazaki Y. The role of the natriuretic peptides in the cardiovascular system. Cardiovasc Res 2001; 51:489–94.
12. Mair J, Hammerer-Lercher A, Puchendorf B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure. Clin Chem Lab Med 2001; 39:571–88.
13. Maisel A. B-type natriuretic peptide levels: a potential novel "white count" for congestive heart failure. J Cardiac Failure 2001; 7:183–93.
14. Chen HH, Burnett JC Jr. Natriuretic peptides in the pathophysiology of congestive heart failure. Cur Cardiol Rep 2000; 2:198–205.
15. Struthers AD. Introducing a new role for BNP: as a general indicator of cardiac structural disease rather than a specific indicator of systolic dysfunction only. Heart 2002; 87:97–8.
16. Remme WJ, Swedberg K. Task Force Report. Guidelines for the diagnosis and treatment of chronic heart failure. Eur Heart J 2001; 22:1527–60.
17. McLaughlin MA, Cassel CK. Demography and epidemiology of cardiovascular disease in old age. In: Chesler E, editor. Clinical cardiology in the elderly, 2nd ed. Armonk: Futura Publishing Company Inc., 1999:3–16.
18. Folsom AR. "New" risk factors for atherosclerotic diseases. Exp Gerontol 1999; 34:483–90.
19. Epstein SE, Zhou YF, Zhu J. Infection and atherosclerosis: emerging mechanistic paradigms. Circulation 1999; 100:e20–8.
20. Smith D, Gupta S, Kaski JC. Chronic infections and coronary heart disease. Int J Clin Pract 1999; 53:460–6.
21. Valtonen VV. Role of infections in atherosclerosis. Am Heart J 1999; 138 (5 Pt 2):S431–3.
22. Siscovick DS, Schwartz SM, Caps M, Wang SP, Grayston JT. Chlamydia pneumoniae and atherosclerotic risk in populations: the role of seroepidemiology. J Infect Dis 2000; 181 (Suppl 3):S417–20.

23. Davydov L, Cheng JW. The association of infection and coronary artery disease: an update. Expert Opin Investig Drugs 2000; 9:2505–17.
24. Fong IW. Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. Can Med Assoc J 2000; 163:49–56.
25. Coppola A, Davi G, De Stefano V, Mancini FP, Cerbone AM, Di Minno G. Homocysteine, coagulation, platelet function, and thrombosis. Semin Thromb Hemost 2000; 26:243–54.
26. Milionis HJ, Winder AF, Mikhailidis DP. Lipoprotein (a) and stroke. J Clin Pathol 2000; 53:487–96.
27. Morrisett JD. The role of lipoprotein[a] in atherosclerosis. Curr Atheroscler Rep 2000; 2:243–50.
28. Kullo IJ, Gau GT, Tajik AJ. Novel risk factors for atherosclerosis. Mayo Clin Proc 2000; 75:369–80.
29. Ridker PM, Genest J, Libby P. Risk factors for atherosclerotic disease. In: Braunwald E, Zipes DP, Libby P, editors. Heart disease: a textbook of cardiovascular medicine, 6th ed. Philadelphia: W.B. Saunders Co., 2001:1010–37.
30. Maron DJ, Ridker PM, Pearson TA, Grundy SM. Dyslipidemia, other risk factors, and the prevention of coronary heart disease. In: Fuster V, Alexander RW, O'Rourke RA, Roberts R, King SB III, Wellens HJJ, editors. Hurst's The heart, 10th ed. New York: McGraw-Hill Medical Publishing Division, 2001:1131–60.
31. Benitez RM, Vogel RA. Assessment of subclinical atherosclerosis and cardiovascular risk. Clin Cardiol 2001; 24: 642–50.
32. Iacoviello L, Vischetti M, Zito F, Donati BM. Genes encoding fibrinogen and cardiovascular risk. Hypertension 2001; 38:1199–203.
33. Emdin M, Passino C, Michelassi C, Titta F, L'abbate A, Donato L, *et al.* Prognostic value of serum gamma-glutamyl transferase activity after myocardial infarction. Eur Heart J 2001; 22:1802–7.
34. Jialal I, Devaraj S. Inflammation and atherosclerosis: the value of the high-sensitivity C-reactive protein assay as a risk marker. Am J Clin Pathol 2001; 116 (Suppl:S):108–15.
35. Kaplan RC, Frishman WH. Systemic inflammation as a cardiovascular disease risk factor and as a potential target for drug therapy. Heart Dis 2001; 3:326–32.
36. Cole DE. Homocysteine as a risk factor in cardiovascular disease. Adv Exp Med Biol 2001; 498:59–64.
37. Gavras I, Gavras H. Angiotensin II as a cardiovascular risk factor. J Hum Hypertens 2002; 16 (Suppl 2): S2–6.
38. Benzaquen LR, Yu H, Rifai N. High sensitivity C-reactive protein: an emerging role in cardiovascular risk assessment. Crit Rev Clin Lab Sci 2002; 39:459–97.
39. Pedrinelli R, Dell'Omo G, Di Bello V, Pontremoli R, Mariani M. Microalbuminuria, an integrated marker of cardiovascular risk in essential hypertension. J Hum Hypertens 2002; 16:79–89.
40. Ford ES, Smith SJ, Stroup DF, Steinberg KK, Mueller PW, Thacker SB. Homocyst(e)ine and cardiovascular disease: a systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. Int J Epidemiol 2002; 31:59–70.
41. Diercks GF, van Boven AJ, Hillege JL, de Jong PE, Rouleau JL, van Gilst WH. The importance of microalbuminuria as a cardiovascular risk indicator: a review. Can J Cardiol 2002; 18:525–35.
42. Mangoni AA, Jackson SH. Homocysteine and cardiovascular disease: current evidence and future prospects. Am J Med 2002; 112:556–65.
43. Dubbert PM, Carithers T, Sumner AE, Barbour KA, Clark BL, Hall JE, *et al.* Obesity, physical inactivity, and risk for cardiovascular disease. Am J Med Sci 2002; 324:116–26.
44. Alderman MH. Uric acid and cardiovascular risk. Curr Opin Pharmacol 2002; 2:126–30.
45. Vanitallie TB. Stress: a risk factor for serious illness. Metabolism 2002; 51 (6 Suppl 1):40–5.
46. Leinonen M, Saikku P. Evidence for infectious agents in cardiovascular disease and atherosclerosis. Lancet Infect Dis 2002; 2:11–7.
47. Dugan JP, Feuge RR, Burgess DS. Review of evidence for a connection between Chlamydia pneumoniae and atherosclerotic disease. Clin Ther 2002; 24:719–35.
48. Hokanson JE. Hypertriglyceridemia and risk of coronary heart disease. Curr Cardiol Rep 2002; 4:488–93.
49. Marian AJ, Roberts R. Molecular basis of hypertrophic and dilated cardiomyopathy. Tex Heart Inst J 1994; 21:6–15.
50. Mestroni L, Krajinovic M, Severini GM, Milasin J, Pinamonti B, Rocco C, *et al.* Molecular genetics of dilated cardiomyopathies. Eur Heart J 1995; 16 (Suppl O):5–9.
51. Marian AJ. Genetic risk factors for myocardial infarction. Curr Opin Cardiol 1999; 13:171–8.
52. Pyeritz RE. Genetic approaches to cardiovascular disease. In: Chien KR, editor. Molecular basis of cardiovascular disease. Philadelphia: WB Saunders Company, 199:19–36.
53. Grossfeld PD, Rothman A, Gruber P, Chien KR. Molecular genetics of congenital heart disease. In: Chien KR, editor. Molecular basis of cardiovascular disease. Philadelphia: WB Saunders Company, 1999:135–65.
54. Seidman CE, Seidman JG. Molecular genetics of inherited cardiomyopathies. In: Chien KR, editor. Molecular basis of cardiovascular disease. Philadelphia: WB Saunders Company, 1999:251–63.
55. Curran ME, Sanguinetti MC, Keating MT. Molecular basis of inherited cardiac arrhythmias. In: Chien KR, editor. Molecular basis of cardiovascular disease. Philadelphia: WB Saunders Company, 1999:302–11.
56. Brugada R, Brugada J, Roberts R. Genetics of cardiovascular disease with emphasis on atrial fibrillation. J Intervent Car Electrophysiol 1999; 3:7–13.
57. Goldmuntz E. Recent advances in understanding the genetic etiology of congenital heart disease. Curr Opin Pediatr 1999; 11:437–43.
58. Pallaud C, Maurice M, Cheng S, Grow M, Aguillon D, Sass C, *et al.* Multilocus approach to cardiovascular risk. Scand J Clin Lab Invest 1999; Suppl 230:168–76.
59. Rapp JP. Genetic analysis of inherited hypertension in the rat. Physiol Rew 2000; 80:135–72.
60. Roberts R, Lifton R. Unraveling the human genome and its future implications for cardiology. In: Fuster V, Alexander RW, O'Rourke RA, Roberts R, King SB III, Wellens HJJ, editors. Hurst's The heart, 10th ed. New York: McGraw-Hill Medical Publishing Division, 2001:147–55.
61. Arbustini E, Morbini P, Pilotto A, Gavazzi A, Tavazzi L. Genetics of idiopathic dilated cardiomyopathy. Herz 2000; 25:156–60.
62. Bowles NE, Bowles K, Towbin JA. Prospects for gene therapy for inherited cardiomyopathies. Prog Pediatr Cardiol 2000; 12:133–45.
63. Towbin JA, Bowles NE. Molecular genetics of left ventricular dysfunction. Curr Mol Med 2001; 1:81–90.
64. Sinagra G, Lenarda A, Brodsky GL, Taylor MR, Muntoni F, Pinamonti B, *et al.* Current perspective new insights into the molecular basis of familial dilated cardiomyopathy. Ital Heart J 2001; 2:280–6.
65. Towbin JA, Vatta M, Li H, Bowles NE. Genetics and cardiac arrhythmias. Adv Pediatr 2002; 49:87–129.
66. Myocardial infarction redefined – a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of

Myocardial Infarction. J Am Coll Cardiol 2000; 36: 959–69.
67. A report of the American College of Cardiology/American Heart Association task force on the practice guidelines. Evaluation and management of chronic heart failure in the adult. ACC/AHA Practice Guidelines 2001.
68. Apple FS. Cardiac function. In: Burtis CA, Ashwood ER, editors. Tietz fundamental of clinical chemistry, 5th ed. Philadelphia: W.B. Saunders Company, 2001:682–97.
69. Grundy SM, Pasternak R, Greenland P, Smith S, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations. A statement for healthcare professionals from American Heart Association and the American College of Cardiology 1999.
70. Benjamin EJ, Smith SC Jr, Cooper RS, Hill MN, Luepker RV. Task force #1-magnitude of the prevention problem: opportunities and challenges. 33rd Bethesda Conference. J Am Coll Cardiol 2002; 40:588–603.
71. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, *et al.* Markers of inflammation and cardiovascular disease. Application to clinical and public health practice. A statement of healthcare professionals from the Center for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107: 499–511.
72. Unger ER, Piper MA. Molecular diagnostics: basic principles and techniques. In: Henry JB, editor. Clinical diagnosis and management by laboratory methods. 20th ed. Philadelphia: W.B. Saunders Company, 2001:1275–86.
73. Schrenzel J, Hibbs JR, Persing DH. Hybridization array technologies. In: Henry JB, editor. Clinical diagnosis and management by laboratory methods. 20th ed. Philadelphia: W.B. Saunders Company, 2001:1296–303.
74. ACC/AHA 2002 guideline update for the Management of Patients with Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction. A report of the American College of Cardiology/American Association task force on practice guidelines. Committee on the Management of Patients with Unstable Angina. American College of Cardiology and the American Heart Association, Inc., 2002:1–95.
75. Christenson RH, Duh SH. Evidence based approach to practice guides and decision thresholds for cardiac markers. Scand J Clin Lab Invest 1999; 59(suppl 230):90–102.
76. Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich KW, Vinar G, Noe A, *et al.* Diagnostic efficiency of troponin T measurements in acute myocardial infarction. Circulation 1991; 83:902–12.
77. Antman EM, Tenasijevic MJ, Thompson B, Schactman M, McCabe CH, Cannon CP, *et al.* Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996; 335:1342–9.
78. Galvani M, Ottani F, Ferrini D, Ladenson JH, Destro A, Baccos D, *et al.* Prognostic influence of elevated values of cardiac troponin I in patients with unstable angina. Circulation 1997; 95:2053–9.
79. Lindahl B, Venge P, Wallentin L, for the FRISC Study Group. Relation between troponin T and the risk of subsequent cardiac events in unstable coronary artery disease. Circulation 1996; 93:1651–7.
80. Luscher MS, Thygesen K, Ravkilde J, Heickendorff L, for the TRIM Study Group. Applicability of cardiac troponin T and I for early stratification in unstable coronary artery disease. Circulation 1997; 96:2578–85.
81. Christenson RH, Duh SH, Apple FS, Bodor GS, Bunk DM, Dalluge J, *et al.* Standardization of cardiac tropinin I assays: round robin of ten candidate reference materials. Clin Chem 2001; 47:431–7.
82. Panteghini M. Recent approaches to the standardization of cardiac markers. Scand J Clin Lab Invest 2001; 61:95–102.
83. Panteghini M. Recent approaches in standardization of cardiac markers. Clin Chim Acta 2001; 311:19–25.
84. Panteghini M. The measurement of cardiac markers. Where should we focus? Am J Clin Pathol 2002; 118:354–61.
85. Galvani M, Panteghini M, Ottani F, Cappelletti P, Chiarella F, Chiariello M, *et al.* New definition of myocardial infarction: analysis of the consensus document ESC/ACC and thoughts about applicability to the Italian health situation. Ital Heart J 2002; 3(9 Suppl):955–70.
86. Pagani F, Bonetti G, Panteghini M. Comparative study of cardiac troponin I and T measurements in a routine extracardiological clinical setting. J Clin Lab Anal 2001; 15:210–4.
87. Kirsh B. Electronmicroscopy of the atrium of the heart. I. Guinea pig. Exp Med Surg 1956; 14:99–112.
88. Jamieson JD, Palade GE. Specific granules in atrial muscle cell. J Cell Biol 1964; 23:151–62.
89. Marie JP, Guillemont H, Hatt PY. Le degré de granulation des cardiocytes auriculaires. Etude planimétriques au cours de differents apports d'eau et de sodium chez le rat. Pathol Biol 1976; 24:549–54.
90. De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and important natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci 1981; 28: 89–94.
91. Clerico A. Pathophysiological and clinical relevance of circulating levels of cardiac natriuretic hormones: Is their assay merely a marker of cardiac disease? [opinion article] Clin Chem Lab Med 2002; 40:752–60.
92. Clerico A, Iervasi G, Pilo A. Turnover studies on cardiac natriuretic peptides: methodological, pathophysiological and therapeutical considerations. Curr Drug Metab 2000; 1:85–105.
93. Nakamura M, Endo H, Nasu M, Arakawa N, Segawa T, Hiramori K. Value of plasma B type natriuretic peptide measurement for heart disease screening in a Japanese population. Heart 2002; 87:131–5.
94. Koglin J, Pehlivanli S, Schwaiblmair M, Vogeser M, Cremer P, von Scheidt W. Role of brain natriuretic peptide in risk stratification of patients with congestive heart failure. J Am Col Cardiol 2001; 38:1934–41.
95. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, *et al.* Utility of B-natriuretic peptide in detecting diastolic dysfunction: comparison with Doppler velocity recordings. Circulation 2002; 105:595–601.
96. Morrison LK, Harrison A, Krishnaswamy P, Kazanegra R, Clopton P, Maisel A. Utility of a rapid B-natriuretic peptide assay in differentiating congestive heart failure from lung disease in patients presenting with dyspnea. J Am Coll Cardiol 2002; 39:202–9.
97. Cabanes L, Richaaud-Thiriez B, Fulla Y, Heloire F, Vuillemard C, *et al.* Brain natriuretic peptide blood levels in the differential diagnosis of dyspnea. Chest 2001; 120:2047–50.
98. Clerico A, Del Ry S, Giannessi D. Measurement of natriuretic cardiac hormones (ANP, BNP, and related peptides) in clinical practice: the need for a new generation of immunoassay methods. Clin Chem 2000; 46:1529–34.
99. Del Ry S, Giannessi D, Clerico A. Plasma Brain Natriuretic Peptide Measured by fully-automated immunoassay and by immunoradiometric assay compared. Clin Chem Lab Med 2001; 39:446–50.
100. Masson S, Vago T, Baldi G, Salio M, De Angelis N, Nicolis

E, *et al.* Comparative measurement of N-terminal pro-brain natriuretic peptide and brain natriuretic peptide in ambulatory patients with heart failure. Clin Chem Lab Med 2002; 40:761–3.
101. Bluestein BI, Belenky A, Lin S, Despres N, Vajdi M, Armstrong G. Development of an automated test for B-type natriuretic peptide (BNP) as an aid in the diagnosis and evaluation of congestive heart failure on the Bayer ADVIA Centaur chemiluminescent system [abstract]. Clin Chem 2002; 48 (Suppl. to 6):A85.
102. Kelly PM, Gaston S, Mackay R, Arthur K, Taylor V, Shih J, *et al.* A novel assay for the measurement of plasma B-type natriuretic peptide by an AxSYM microparticle based immunoassay with use of stable liquid calibrators [abstract]. Clin Chem 2002; 48 (Suppl. to 6):A94.
103. Clerico A, Del Ry S, Maffei S, Prontera C, Emdin M, Giannessi D. Circulating levels of cardiac natriuretic hormones in healthy adult subjects: effects of aging and sex. Clin Chem Lab Med 2002; 40:371–7.
104. Wang TJ, Larson MG, Levy D, Leip EP, Benjamin EJ, Wilson PW, *et al.* Impact of age and sex on plasma natriuretic peptide levels in healthy adults. Am J Cardiol 2002; 90:254–8.
105. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002; 40:976–82.
106. Vasan RS, Benjamin EJ, Larson MG, Leip EP, Wang TJ, Wilson PW, *et al.* Plasma natriuretic peptides for community screening for left ventricular hypertrophy and systolic dysfunction. J Am Med Assoc 2002; 288:1252–9.
107. Nakamura M, Endo H, Nasu M, Arakawa N, Segawa T, Hiramori K. Value of plasma B type natriuretic peptide measurement for heart disease screening in a Japanese population. Heart 2002; 87:131–5.
108. Libby P. The vascular biology of atherosclerosis. In: Braunwald E, Zipes DP, Libby P, editors. Heart disease, 6th ed. Philadelphia: W.B. Saunders Company, 2001:995–1009.
109. Massey DH, McPherson RA. Cytokines and adhesion molecules; In: Henry JB, editor. Clinical diagnosis and management by laboratory methods, 22th ed. Philadelphia: W.B. Saunders Company, 2001:914–26.
110. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340:115–26.
111. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105:1135–43.
112. Dollery CM, McZwan JR, Henney AM. Matrix metalloproteinases and cardiovascular disease. Circ Res 1995; 77:863–8.
113. Zucker S, Hymowitz M, Conner C, Zarrabi HM, Hurewitz AN, Matrisian L, *et al.* Measurement of matrix metalloproteinases and tissue inhibitors of metalloproteinases in blood and tissues. Clinical and experimental applications. Ann N Y Acad Sci 1999; 878:212–27.
114. Lindsay MM, Maxwell P, Dunn FG. TIMP-1: a marker of left ventricular diastolic dysfunction and fibrosis in hypertension. Hypertension 2002; 40:136–41.
115. Kalela A, Laaksonen R, Lehtimaki T, Koivu TA, Hoyhtya M, Janatuinen T, *et al.* Effect of pravastatin in mildly hypercholesterolemic young men on serum matrix metalloproteinases. Am J Cardiol 2001; 88:173–5.
116. Lindholt JS, Vammen S, Fasting H, Henneberg EW, Heickendorff L. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms. A preliminary study. Eur J Vasc Endovasc Surg 2000; 20:281–5.
117. O'Brien ER, Garvin MR, Dev R, Stewart DK, Hinohara T, Simpson JB, *et al.* Angiogenesis in human coronary atherosclerotic plaques. Am J Pathol 1994; 145:883–94.
118. Andreassi MG, Botto N, Rizza A, Colombo MG, Palmieri C, Berti S, *et al.* Deoxyribonucleic acid damage in human lymphocytes after percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 2002; 40:862–8.
119. Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, *et al.* Evidence for DNA damage in patients with coronary artery disease. Mutat Res 2001; 493:23–30.
120. Forrester JS. Prevention of plaque rupture: a new paradigm of therapy. Ann Intern Med 2002; 137:823–33.
121. Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K, *et al.* Oxidized LDL in carotid plaques and plasma associates with plaque instability. Arterioscler Thromb Vasc Biol 2002; 22:1649–54.
122. Albertini R, Moratti R, De Luca G. Oxidation of low-density lipoprotein in atherosclerosis from basic biochemistry to clinical studies. Curr Mol Med 2002; 2:579–92.
123. Libby P, Simon DI. Inflammation and thrombosis. Circulation 2001; 103:1718–20.
124. Theroux P, Fuster V. Acute coronary syndrome: unstable angina and non-Q-wave myocardial infarction. Circulation 1998; 97:1195–206.
125. Galvani M, Ferrini D, Ottani F, Eisenberg PR. Early risk stratification of unstable angina/non-Q myocardial infarction: biochemical markers of coronary thrombosis. Int J Cardiol 1999; 68 (Suppl 1):S55–61.
126. Tripodi A, Mannucci PM. Laboratory investigation of thrombophilia. Clin Chem 2001; 47:1597–606.
127. Peters RJG. Gene polymorphisms and the risk of myocardial infarction. An emerging relation. N Engl J Med 2002; 347:1963–5.
128. Reich DE, Lander ES. On the allelic spectrum of human disease. Trends Genet 2001; 17:502–10.
129. Andreassi MG, Botto N, Colombo MG, Biagini A, Clerico A. Genetic instability and atherosclerosis: can somatic mutations account for the development of cardiovascular diseases? Environ Mol Mutagen 2000; 35:265–9.
130. Towbin JA, Roberts R. Cardiovascular diseases due to genetic abnormalities. In: Fuster V, Alexander RW, O'Rourke RA, Roberts R, King SB III, Wellens HJJ, editors. Hurst's The heart, 10th ed. New York: McGraw-Hill Medical Publishing Division, 2001:1785–835.
131. Clark EB. Etiology of congenital cardiovascular malformations: epidemiology and genetics. In: Allen HD, Gutgesell HP, Clark EB, Driscoll DJ, editors. Heart disease in infants, children, and adolescents, 6th ed., vol. 1. Philadelphia: Lippincott Williams & Wilkins, 2001:64–79.
132. Bing RJ. Preface. In: Bing RJ, editor. Cardiology – the evolution of the science and the art, 2nd ed. Chur (Switzerland): Harwood Academic Publishers GmbH, 1999:XIII–XIV.

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