Imaging and Laboratory Biomarkers in Cardiovascular Disease

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Abstract: Imaging and laboratory biomarkers are an essential support to modern practice of medicine, allowing a better identification, severity titration, staging and follow-up of atherosclerosis and heart failure disease. This review provides an overview of imaging, biochemical and genetic biomarkers used in clinical practice and for research purposes in order to evaluate the 4 different aspect of patient vulnerability to cardiovascular disease: arterial; blood; myocardial; metabolic vulnerability.

Yet, no single perfect biomarker exists and there is wide room for optimization and integration between clinical evaluation and biomarker evaluation. In general, a targeted approach tailored on the individual patient should be preferred to a carpet diagnostic bombing, which will lead to an exorbitant multiplier of costs, risks and inappropriate testing.

Key Words: Atherosclerosis, biomarkers, cost, risk.

INTRODUCTION

In 2001, a National Institute of Health working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to therapeutic interventions" [1]. The desirable properties of biomarkers vary with their intended use. Proper biomarkers can be used in therapeutic trials as indicators or substitutes for clinically meaningful endpoints. They can serve as surrogate clinical endpoints and provide a direct measure of how the patient feels, functions or survives and can predict the effects of a therapy. Biomarkers can replace distal clinical endpoints with more proximal ones that can be measured earlier, more frequently and with higher precision. Biomarkers may require reduced sample sizes and allow for faster decision making in incorporating or eliminating newer therapeutic approaches. Regardless of the purpose for its use, a new biomarker will be of clinical value only if it is accurate, it is reproducibly obtained in a standardized fashion, it is acceptable to the patient, it is easy to interpret by clinicians, it has high sensitivity and specificity for the outcome it is expected to identify, and there are data to suggest that knowledge of biomarkers levels changes management [2]. Many biomarkers in cardiovascular disease fulfil these desirable properties, and are therefore used in clinical practice and suitable candidates in cardiovascular clinical trials. They can be distinguished into imaging, biochemical (laboratory) and genetic biomarkers. Examples of imaging biomarkers include the degree of left ventricular dysfunction in heart failure, the carotid plaque burden in atherosclerosis, the extent and severity of stress-induced myocardial ischemia in coronary artery disease [2]. Examples of laboratory biomarkers include the NT-proBNP and troponin, established clinical markers of heart failure and myocardial tissue damage respectively.

Regarding genetic biomarkers, common genetic polymorphisms (Factor V Leiden and 20210G>A prothrombin mutations) are currently example of genetic factors used into clinical practice for screening the risk for thrombosis [3].

The variety of new biomarkers, the rapid evolution in the field, the uncertainty about the role of the new technology and insecurity regarding the current standard in the area led to substantial controversy over which biomarker should be used, in which patient, and when. It is also unclear if there is a synergistic rather than antagonistic role of the 2 types of biomarkers. The potential usefulness of such synergism is highlighted by the current and rumoured future acquisition of several biomarker companies by major imaging companies [4]. On the other side, there are clear risks that this marketing driven synergism may multiply the already worrisome high levels of inappropriateness in the field of imaging and laboratory testing [3,5-9]. The roadmap to validation of biomarkers follows 4 regulatory steps: stage 1 ("promising" pre-biomarkers) or 2 ("developing" biomarker, with known accuracy and reproducibility under highly controlled conditions) to either one of 2 possible destinies: dismissal-downgrading (which will limit/close further clinical use reducing costs and development time) or upgrading to fully exploitable stage 3 ("developed" biomarker with established value added in a given clinical setting) or 4, i.e. "validated gatekeeper" required and/or accepted to assure safety and/or efficacy of an approved/licensed therapeutic intervention [1]: (Fig. **1**). Only a low percentage of imaging candidates entering the biomarker development process will eventually survive to the extensive medical and research application stage. Examples of markers, which never reached the role of gatekeeper – in spite of promising early results – are the use of echocardiography predictors in identifying responders to cardiac resynchronisation therapy on the basis of mechanical dyssynchrony [10] or myocardial perfusion contrast echo-

*Address correspondence to this author at the CNR, Institute of Clinical Physiology, Via Moruzzi, 1, 56124 Pisa, Italy; Tel: +39 – 050-3152400; Fax: +39-050-3152374; E-mail: picano@ifc.cnr.it

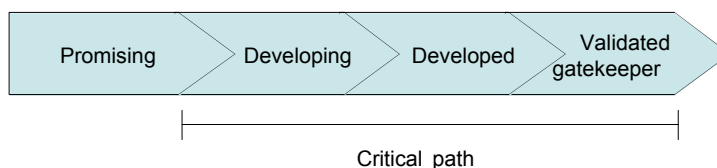# New biomarkers for new pathophysiological targets

Fig. (1). The natural history of a biomarker, from the seed of efficacy to the fruit of effectiveness.

cardiography for detection of coronary artery disease [11]. However, an impulse to this field is expected from the general interest -shared by academia, pharma industry and major regulatory bodies- to promote the qualification of molecular imaging as a biomarker in drug developmen,t in response to the relevant attrition and diverging costs emerged in the pipeline of new medicine discovery and validation [e.g. http: //imi.europa.eu/index_en.html; http: //www.fda.gov/oc/initiatives/criticalpath/].

Clinicians, scientists and the industry need an early and accurate identification of imaging and laboratory biomarkers of proved medical value and methodological robustness. The seed of efficacy under controlled clinical conditions should not be mistaken for the fruit of effectiveness, i.e. the value of the technique when deployed in the field [12].

THE PATHOPHYSIOLOGICAL TARGETS OF BIOMARKERS IN CARDIOVASCULAR DISEASE

The different biomarkers focus on different aspect of patient vulnerability to cardiovascular disease: arterial; blood; myocardial; metabolic vulnerability. All 4 vulnerabilities concur to the clinical expression of atherosclerotic disease and heart failure, which may occur as a consequence of and independently from atherosclerotic disease (Fig. **2**).

Acute coronary syndromes are accompanied by progressive mechanical obstruction, dynamic obstruction, and plaque inflammation, instability, and rupture, followed by superimposed thrombosis – which is also determined by the pro-thrombotic tendency of blood: the "*vulnerable blood*". Imaging can help marginally in the identification of low flow states, for instance by spontaneous echocontrast by TTE or reduced left atrial appendage flow velocity by Doppler TEE during atrial fibrillation, which identify a high risk of impending thrombosis [13]. Once plaque-superimposed thrombosis occurs, it may be manifest as elevation of circulating D-dimer, plasminogen activator inhibitor-1 and von-Willebrand factor.

The vulnerable myocardium can be identified through structural or functional markers. Among structural markers, left ventricular hypertrophy or left ventricular concentric remodelling (with normal left ventricular mass) can be best identified with real time 3D echocardiography [14]. Functional markers are based on stress-induced perfusion (or coronary flow reserve) abnormalities, mirroring a coronary microvascular abnormality which may identify early stages of incipient cardiomyopathy, even with normal coronary arteries [15]. At a more advanced stage of disease, myocardial vulnerability can be identified as left ventricular dysfunction (by ultrasound, CMR, CT or nuclear scans).

Arterial vulnerability can be tracked again with functional markers (such as endothelial dysfunction evaluated with brachial artery reactivity testing or by myocardial PET imaging, the latter directly measuring coronary responses in a quantitative fashion and structural markers (such as carotid intima-media thickness by ultrasound and coronary artery calcium by CT) [16-18].

An additional, emerging dimension is the *metabolic vulnerability*. Along the traditional risk factors such as total cholesterol, LDL-C, low HDL, and glycemia, recently new factors were put forward such as gamma-GT [19], adiponectin, leptin [20], and fT3 [21]. Laboratory biomarkers are more informative than bioimaging markers in identifying metabolic vulnerability. The possibility of imaging visceral fat (by CMR, CT or – to some extent – ultrasound) is however a novel, exciting perspective [22].

ATHEROSCLEROSIS CASCADE

The research interest and potentially appropriate clinical application of biomarkers are best framed within the current understanding of the pathobiology of atherosclerosis and heart failure. For atherosclerosis (Fig. **3**), endothelial dysfunction (a functional imaging marker of arterial vulnerability) or pre-intrusive atherosclerosis (a structural imaging marker, commonly detected as increased carotid intima-media thickness) may progress over decades to impairment in coronary flow reserve, and only later to stress-induced dysfunction and eventually to less reversible stress-induced dysfunction at rest [23].

The time period preceding the onset of an acute coronary syndrome is characterized by atherosclerotic arterial lesions prone to rupture. Such lesions are rich in macrophages (which release lytic enzymes like metalloproteinases) and are associated with a reduction in smooth muscle cells, increase in lipids, and a thin fibrous cap [24]. These biochemical characteristics of vulnerable plaques are major targets for the development of specific tracers based on peptides for the detection of vascular remodelling [25,26] and glucose metabolism for inflammation assessment, due to the avidity of macrophages for this substrate[27,28]. Acute coronary syndromes are accompanied by progressive mechanical dys-
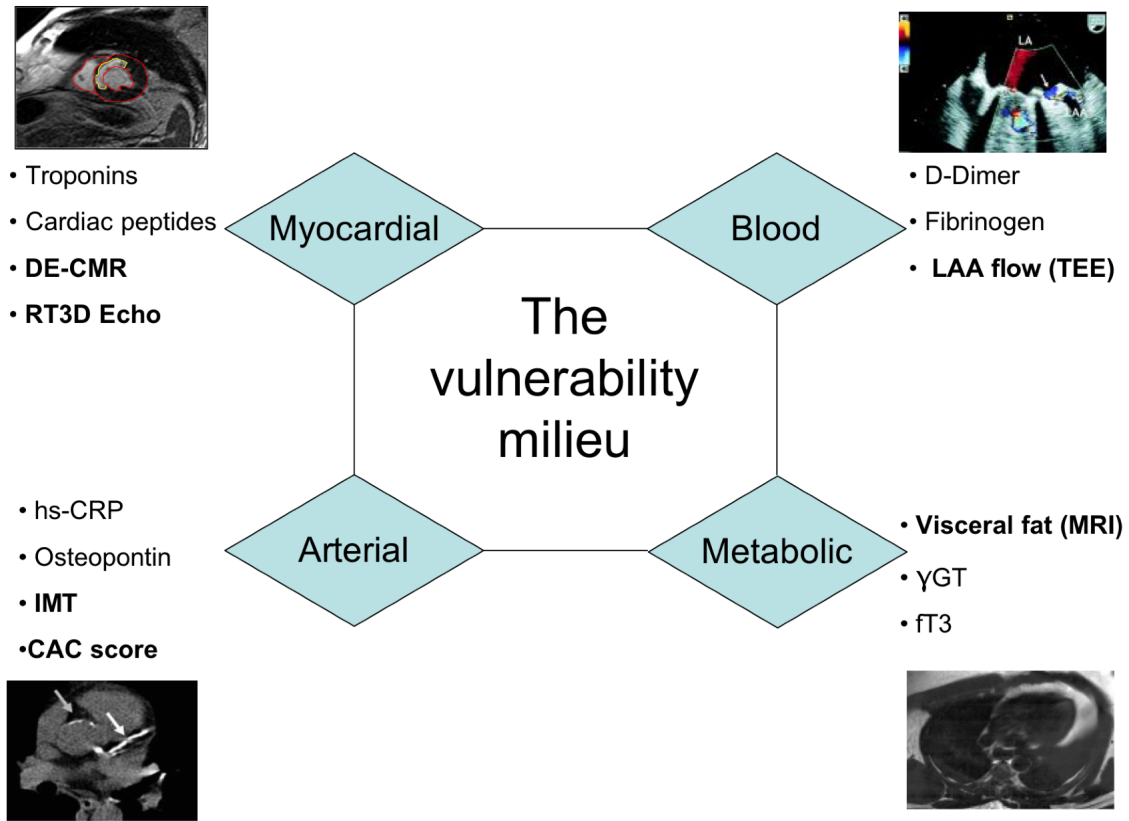
- Troponins
- Cardiac peptides
- **DE-CMR**
- **RT3D Echo**

Myocardial

Blood

- D-Dimer
- Fibrinogen
- **LAA flow (TEE)**

The vulnerability milieu

- hs-CRP
- Osteopontin
- **IMT**
- **CAC score**

Arterial

Metabolic

- **Visceral fat (MRI)**
- γGT
- fT3

Fig. (2). The vulnerable milieu of the cardiovascular patient. For each of the 4 vulnerabilities, some commonly employed bioimaging (bold) and biochemistry markers are shown.

Biomarkers and atherosclerosis

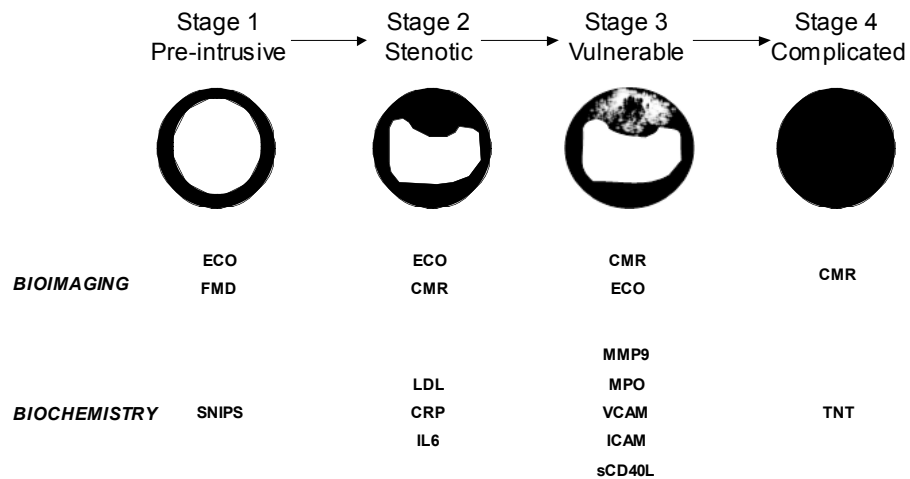|	Stage 1 Pre-intrusive	Stage 2 Stenotic	Stage 3 Vulnerable	Stage 4 Complicated
BIOIMAGING	ECO FMD	ECO CMR	CMR ECO	CMR
BIOCHEMISTRY	SNIPS	LDL CRP IL6	MMP9 MPO VCAM ICAM sCD40L	TNT

Fig. (3). The atherosclerosis cascade.

function, dynamic obstruction, and plaque inflammation, instability, and rupture, followed by superimposed thrombosis. Superimposed thrombosis may be manifest as elevations of circulating D-dimer, plasminogen activator inhibitor 1, and von Willebrand factor. Ischemia is antedated by release of ischemia-modified albumin by a few hours and the development of myocardial necrosis by time-dependent release of myocytes components such as troponins [29]. The hemodynamic consequences of ischemia and/or infarction are reflected by elevation of plasma natriuretic peptide levels. It is therefore obvious that there is no "one marker fits all" approach, but rather the targeted marker tailored in a given patient in a certain clinical theatre: emergency department, physician's office, coronary care unit.

The functional stage of coronary endothelial dysfunction is at present best characterized by pharmacological vasodilation, and this can be quantitatively assessed by myocardial PET imaging [18]. A simpler and more accessible, indirect index is represented by flow-mediated dilation of brachial artery by ultrasound, even though peripheral and coronary responses do not always correlate.

The functional stage of endothelial dysfunction is at present best characterized by flow mediated dilation of brachial artery by ultrasound. There is uncertainty regarding the clinical role of coronary calcium score by CT. Early, asymptomatic atherosclerosis has a skeleton in its closet, and the amount of coronary calcium correlates moderately closely to the overall atherosclerotic plaque burden, but its place in risk stratification of asymptomatic individuals remains uncertain. It is true that the use of calcium score has quite convincing (non randomized) outcome impact over years [30], but it is equally true that the open question remains on how much incremental information can be obtained by CT angio [31]. It is also unclear if the extra-information provided by calcium score assessment is better than the one provided by simpler, radiation-free atherosclerosis imaging biomarkers such as carotid intima-media thickness by ultrasound scan [32]. Recent guidelines do not recommend unselected "screening" or patient self-referral in individuals with very low (<1.0% annual risk) or very high risk (>2% annual risk). A beneficial contribution can most likely be expected in individuals who seem to be at intermediate risk for coronary events (1.0-2.0% annual risk), yet this hypothesis has not been prospectively studied so far [33].

The real disruptive technology appeared in the last 5 years is multi-slice CTA. The information content of CTA is very high, in any case higher than invasive angiography, probably equivalent to gray scale intravascular ultrasound plus angiography. Invasive coronary angiography can identify obstructive as well as complex lesions, but it is restricted to the coronary lumen and is unable to visualize the coronary wall. Thus, features as vessel remodelling or plaque composition are missed. CTA depicts not only a coronary luminogram as coronary angiography does, but also the thickness of the wall and the plaque composition to some extent [34] as ultrasound does [35,36]. This is especially important in the early diagnosis of coronary artery disease, since the earliest stage of atherosclerosis is the initial positive remodelling with preserved lumen, as plaque accumulates [37] (Fig. **1**). Several studies showed an increased level of inflammatory markers, high lipid cores and pronounced medial thinning in positively remodelled vessels [38]. Some of the initial acute presentations of the disease may occur when the adaptive remodelling mechanisms are exhausted and a threshold mass of plaque (depending on vessel diameter) starts to breach towards the lumen. CTA can also offer an insight on plaque structural composition which – for any given plaque size – contribute to plaque vulnerability [10], with lipid rich, high-risk plaques (hypoechoic by ultrasound and hypodense by CT), more prone to rupture and subsequent thrombotic occlusion than calcium rich, low-risk plaques (hyperechoic with shadowing by ultrasound and hyperdense by CT). There is growing evidence that hybrid imaging by PET (coronary flow reserve) and CTA may provide a more conclusive evaluation, especially in medium risk

patients [39]. However, the main constraint of CTA and PET-CTA is exposure to ionizing radiation [40] that limits any screening application policy [41,42]. The forthcoming perspective [43] of hybrid PET-MRI and the increasing sensitivity of PET scanners may change to some extent this aspect.

HEART FAILURE CASCADE

The ageing of the population combined with the improved post-infarction survival has rapidly increased the number of patients with heart failure with a prevalence estimated as much as 1-2 % of the general population that rises to 10% in the elderly (Fig. **4**).

In formulating the 2001 document, ACC/AHA guidelines developed a new approach – also endorsed in the 2005 document – to the classification of heart failure, identifying 4 stages [44]: stage A (at high risk, but without structural heart disease, e.g. hypertension); stage B (structural heart disease but without signs and symptoms of heart failure, e.g. previous myocardial infarction or asymptomatic valvular heart disease); stage C (structural heart disease with current or prior symptoms of heart failure) and stage D (refractory heart failure requiring specialized interventions). According to this staging approach, that is conceptually similar to that achieved by staging in other diseases such as cancer, patients would be expected to either not advance at all or to advance from one stage to the next, unless progression of the disease was slowed or stopped by treatment. Structural changes in Stage B heart failure patients may involve the assessment of cardiac remodelling and hypertrophy as well as myocardial fibrosis. Left ventricular hypertrophy and left ventricular remodelling are best studied with real time 3-D echocardiography, more accurate and reproducible, as well as less time consuming, than 2D echo. CMR can provide – at a higher cost – an equally reliable information, and has also the unique capability to provide an accurate estimate of the presence, transmural extent, and severity of myocardial fibrosis with delayed enhancement protocols [45]. Among biochemistry markers, matrix metalloproteinase and their counter-regulatory inhibitors have been studied with promising results. In presence of congestive heart failure, natriuretic peptides have a major role. They can be helpful in distinguishing heart failure from other causes of dyspnoea [46]. Increases in cardiac natriuretic peptides and of biomarkers of cardiac imaging can also define higher and lower risk subsets of patients with heart failure [47,48].

GENETIC MARKERS: AT THE SOURCE OF THE CASCADE

Recent advances in genomic, proteomics and other "omics" fields promise the discovery of new biomarkers raising the perspective of "personalised medicine".

Genomics-based diagnostics will be incorporated into clinical practice, and they are used to diagnose, predict disease onset or recurrence, tailor treatment options, and assess treatment response.

Genetic biomarkers are defined as variants in the DNA code that alone or in combination are associated with disease expression, disease susceptibility, and disease outcome, including therapeutic response. Some genetic findings are be-

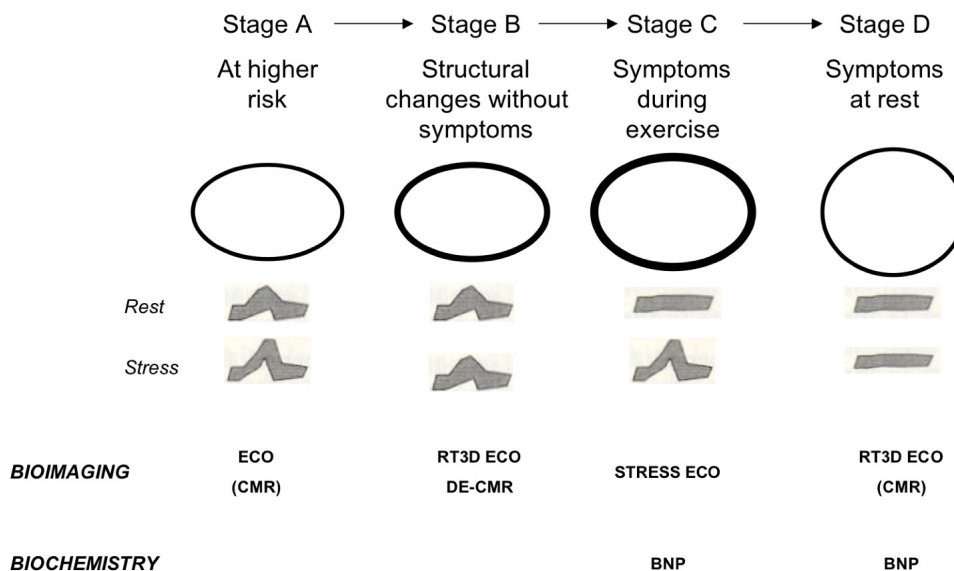# The heart failure cascade

Fig. (4). The heart failure cascade. First row: the structural imaging markers; second row: the functional imaging markers.

ginning to play an important role in the cardiovascular medicine.

For instance, significant advances have been made in recent years in identifying the genetic basis of a variety of monogenic cardiovascular diseases, including familial cardiomyopathies [49-52].

Subsequently, genetic tests have been developed for the major mutations responsible for the disease and are used to confirm the diagnosis and to screen potentially affected relatives.

Uniformly, inherited cardiomyopathies are characterized by a marked phenotypic and genetic heterogeneity, with incomplete and age-dependent penetrance. Genotype-phenotype relations are complex and not yet completely understood. Over 700 mutations in 11 genes encoding contractile proteins of the cardiac sarcomere have been associated with hypertrophic cardiomyopathy (HCM).

Routine, extensive, clinical DNA testing is, at present. impractical because it is costly and time-consuming, but some features, such as ventricular septum morphology, electrocardiogram parameters, and additional clinical parameters can help target DNA analysis of specific genes. [51,52]. For practical purposes, clinical testing is generally performed on the 3 most frequently involved sarcomere genes (*β*-myosin heavy-chain, *MYH7*; myosin-binding protein C, *MYBPC;* and cardiac troponin T,*TNNT2*), estimated to account for about 60% of all familial clinically overt HCM forms.

Familial dilated cardiomyopathy (DCM) is also characterized by high genetic heterogeneity as mutations in >20 genes have been associated with the disease [49-52]. Despite the high number of disease genes, lamin A/C (*LMNA*) gene mutations (~30-40 % of cases) are the most frequent cause of familial DCM cases with cardiac conduction disorders, and they are correlated with a worse prognosis, particularly of

sudden cardiac death and ventricular arrhythmias [51-53]. Therefore, genetic testing for LMNA in DCM patients and families is recommended for preventing fatal events. It is expected that ongoing genetic studies in validating the genotype–phenotype correlations will increase the clinical use of genetic markers in order to improve the clinical management of affected patients as well as to help the screening of family members.

However, the greatest promise and power of genomics-based diagnostics is to identify a relevant set of causative genes for common multigenic disorders, such as atherosclerosis and heart failure.

In these types of disorders, the inherent genetic component mainly result from a single nucleotide polymorphisms (SNPs) in the gene that can affect the concentration or the function of the resulting translated protein. Specific SNPs may determine an individual's susceptibility to disease development and progression and/or the potential response to drug treatments.

Factor V Leiden (R506Q) and prothrombin (20210G>A) mutations provide the best example of how genetic biomarkers may be put into practice in the next future [9].

With advances in technology and additional knowledge, other SNP testing will soon become a routine part of clinical practice.

In actual fact, SNPs on chromosome 9, that have been consistently shown to be associated with atherosclerosis development and progression, may represent the most promising genetic markers to predict the individual cardiovascular risk [54].

Functional genetic variants can be responsible for imaging alterations. There are also examples of gene variants that affect the imaging phenotype under stress condition. For instance, Arg389Gly polymorphism of β_1- adrenoreceptors

affect the contractile response detectable by dobutamine stress echo: homozygous Arg389-β_1 AR subjects exhibited larger inotropic and blood pressure responses than subjects carrying one or two Gly389 alleles. [55].

Other studies have reported that family screening of pulmonary artery systolic pressure (PASP) response to exercise may lead to an earlier diagnosis and more effective therapy for familial form of primary pulmonary hypertension (PPH). In fact, it has been observed that asymptomatic carriers of bone morphogenetic protein receptor type-2 (BMPR2) gene mutation in families with heritable PPH may show an abnormal increase in pulmonary artery systolic pressure during exercise stress echocardiography [456]: (Fig. **5**).

OPEN ISSUES 1: COSTS AND RISKS OF BIOMARKERS

Barriers to introducing new test are not restricted to lack of evidence of efficacy. Safety, tolerability, difficulty in performing the exam and finally cost are important determinants in the choice of the biomarker in the clinical practice.

One of the prerequisites for the clinical use of biomarkers is demonstration of cost-effectiveness and risk-effectiveness. In fact, different biomarkers have different costs and some of them are not without risks. The cost of different imaging techniques is listed in Fig. (**6**) [57]. It is shown that PET can be equally, or even more, accurate than SPECT, but its clinical use is limited by relatively high costs and complexity. Similarly, CAC score or – mostly – noninvasive coronary angiography by CT are fascinating techniques, but their use as screening procedures has been slowed by radiation dose and associated long-term risks (Fig. **7**) [58]. Cost considerations should also be considered for laboratory biomarkers. Although some evidence has been collected for instance with BNP in acute dyspnoea [59] and troponin in acute chest pain [60] in the emergency setting, usually information on cost is conspicuously lacking in scientific publication and clinical prescription patterns. In the use of one biomarker over the other, the cost of complementary or competing techniques should be considered. For instance, based on 2004 Medicare reimbursement data, each BNP test costs less than one eighth
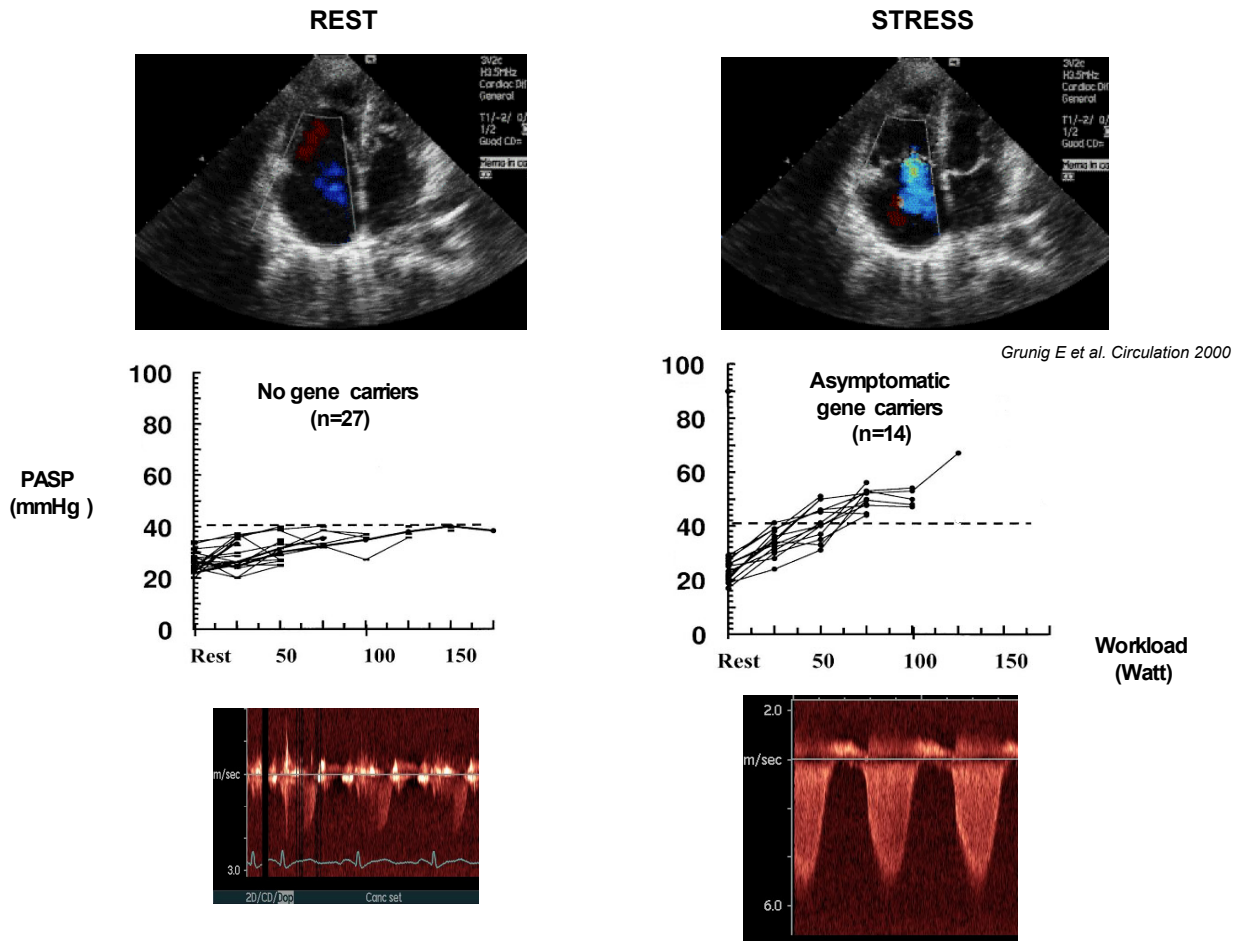

Fig. (5). An example of genetic imaging: exercise stress echo in carriers of abnormal genes of pulmonary hypertension in the pre-clinical, asymptomatic phase. Abnormal response of PASP to exercise in gene carriers. All family members with exercise-induced pulmonary hypertension (AR group, right) were carriers of the disease-associated mutation. In contrast, only 2 of the 27 members (black lines, filled squares) with normal PASP response to exercise (NR group, left) had the disease-associated mutation. Resting PASPs were normal in both groups (mean PASP, 23.3±4 mm Hg vs 24±4 mm Hg in abnormal response vs normal response members, respectively; P=0.57). PASP estimated by Doppler echocardiography was significantly different between abnormal and normal response members during supine bicycle exercise. Modified by ref. [56].

The cost of imaging
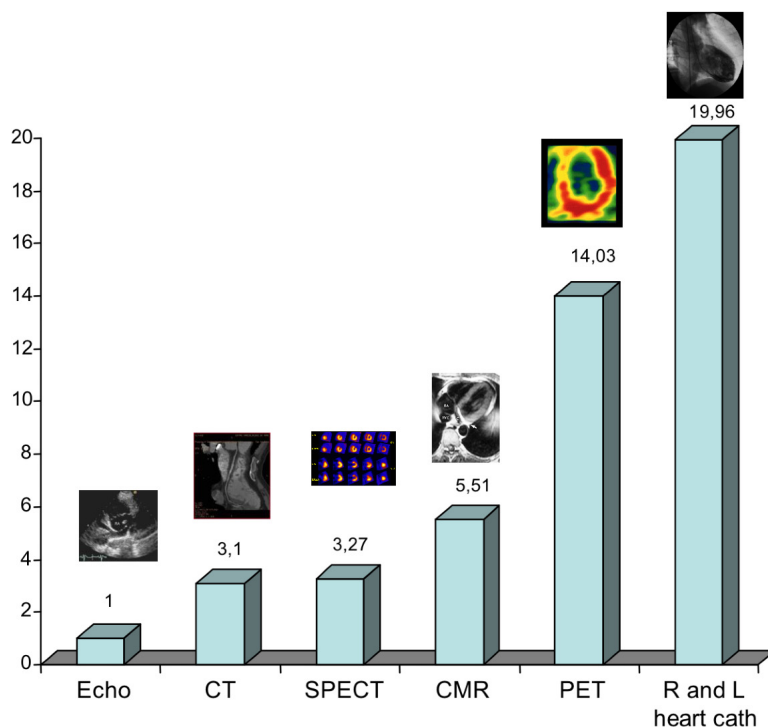
Fig. (6). Estimated average costs of common cardiac imaging procedures when compared to 2D echocardiography. Modified by ref. 57

as much as a Doppler echo examination. The clinician should also consider, however, that the information of an imaging test such a resting echocardiogram extends well beyond the ruling out of a cardiac origin of dyspnoea, and includes unique data on left ventricular structure, function, valvular disease, pericardium, aorta, right ventricle, and pulmonary hemodynamics, and now extravascular lung water [61] – which most directly helps in identifying the cause of dyspnea [62].

Other considerations should also be accounted for laboratory biomarkers. New tests, like the measurement of adiponectin or IL6, are often not standardized and not designed for routine analyses. For this reason, they are also very expensive with the price dependent on the type of assay used for the measurements. At current, these tests are offered only in research facilities and not available for routine analyses. Recently, traditional routine laboratory exams, like GGT and urinary albumin, were considered among the biomarkers for CVD risk [63, 64]

However, the risk increment captured by elevated levels of new markers is modest, and little improvement is seen in traditional measures of discrimination such as the C statistic or the area under the receiver- operating-characteristic curve. [65].

As regard genetic markers, they basically differ from other chemical biomarkers. Genetic tests remain generally expensive technologies that are labour-intensive and time-consuming.

Costs of genetic tests may vary considerably (from several hundreds to thousands Euro €) depending on the number of genes and nucleotides examined. For testing gene the first family member, sequencing gene now cost on the order of €1500-€4000. If a mutation is identified, other family members may be offered confirmatory testing at a reduced rate that is around €250.

On the other hand, there is considerable debate over the new ''predictive medicine'' that could generate serious ethical, social and psychological consequences. Receiving genetic risk information may, therefore, be more harmful than positive by raising unnecessary anxieties and providing a real prospect of discrimination based on a person's genetic make-up [9].

Therefore, genetic testing is not appropriate for every patients, but it should be used in selected cases, such as patients with an established family history of cardiomyopathy in ≥2 closely related relatives or in at-risk patients, when effectively can provide useful information for clinical management.

OPEN ISSUES 2: THE MULTIMARKER APPROACH

Information experts know very well that too much information can be equally uninformative as lack of information.

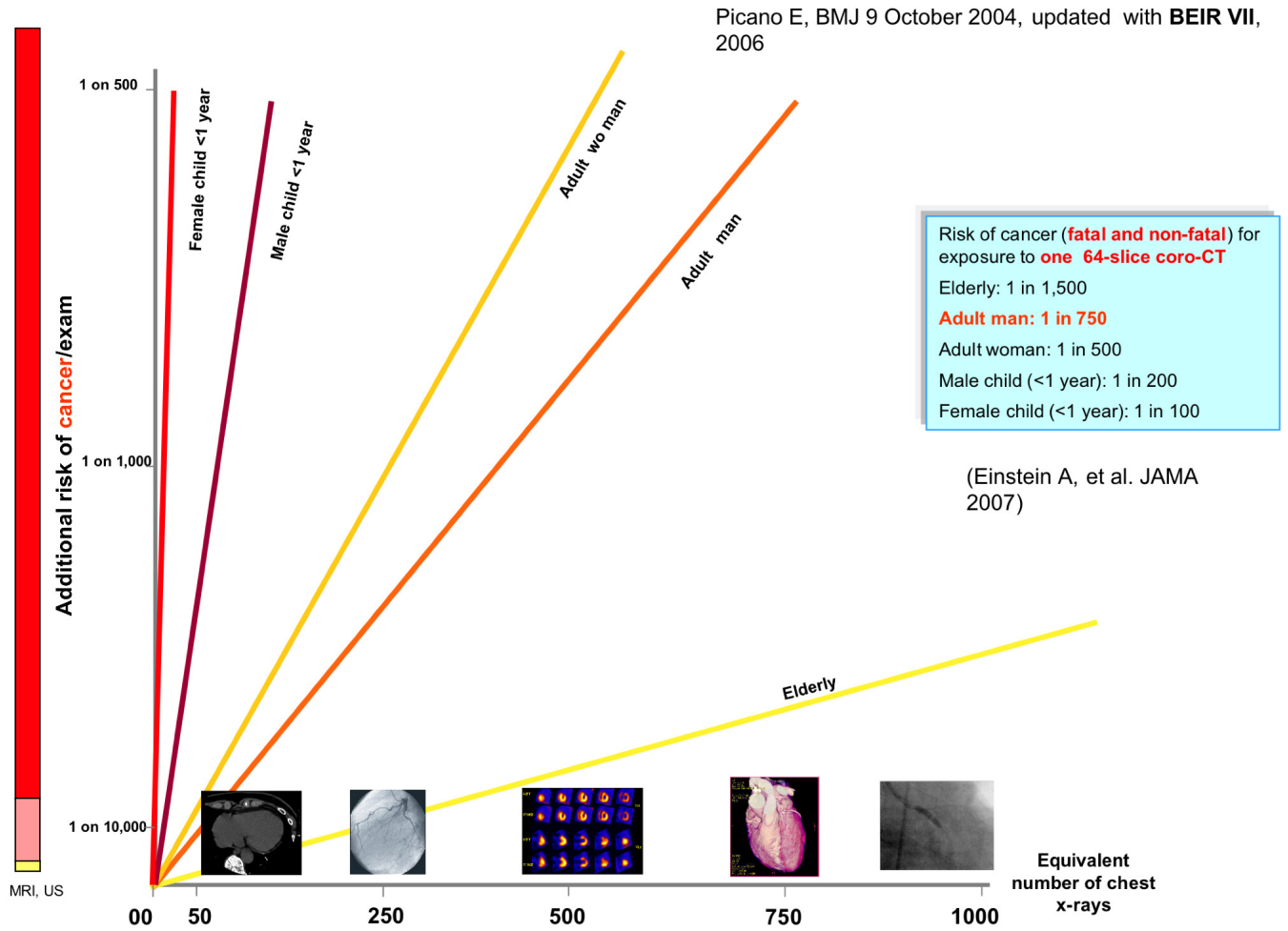**Fig. (7).** Risk stratified according to age and gender. The risk is 37% higher in women than in men, and 4-fold higher in children <1 year than in adults. The risk is reduced by one-half in elderly (>80 years). Redrawn and modified from ref. 58, on the basis of novel estimates of BEIR VII.

Areas for redundancies, contraindications, mistakes, and – in a word – noise are magnified as the number of sources of information increase. Many markers do explore basically the same aspect of vulnerability and the same stage of disease. For instance, inflammation plays a pivotal role in athero-genesis, rapid coronary artery disease progression, plaque disruption and the acute clinical manifestation that result from intracoronary thrombus formation [66]. The most ex-tensively investigated and accessible inflammatory marker for clinical use is CRP, which shows conclusively demon-strated prognostic implications in patients with acute coro-nary syndromes, stable coronary disease, and even in appar-ently healthy men and women in whom CRP is an independ-ent predictor of risk for myocardial infarction and stroke [67]. Other proposed inflammatory markers include neopro-tein, cytokines, adhesion molecules, white blood cell count, CD40L, E-selection, Von Willebrand factor, pregnancy-associated plasma protein A, lipoprotein associate phos-phorilase A2, and neutrophil myeloperoxidase [2,67]. Simi-lar conceptual overlap is present for markers of plaque rup-ture (sCD40L, PIGF, PAPP-A, VCHM), thrombosis (PAI-1, sCD40L, VnF, D-dimer), ischemia (ischemia-modified al-bumin, free fatty acids, choline), necrosis (troponin T, tro-ponin I, CK-MB, myoglobin) and son on. The picture is likely to be further complicated by the advent of "omic" markers and the incorporation of bioimaging data in the theoretically sound "multi-marker approach" based on the statistical analysis of several biomarkers together. An "inde-pendent risk factor" should be defined as a biomarker that retains its statistical association with the outcome when other established risk factors (predictors) for the outcome are in-cluded in the model – but cardiovascular biomarkers are of-ten correlated to each other. The mind-set "more is better" approach produces surprisingly little clinical and prognostic incremental benefits. We need robust statistical models im-proved by the bioengineering of complexity (coping with uncertainties) to produce simple tools for the busy clinician [68, 69].

OPEN ISSUES 3: THE QUEST OF NOVEL BIO-MARKERS

We need better markers in many difficult clinical condi-tions. The vulnerable plaque can be imaged with a variety of techniques providing insight into the structural components at the basis of its own vulnerability, such as thin fibrous cap

and high lipid core. Still, we miss a direct imaging of atherosclerotic plaque activation, which is in the focus of high-specificity radiotracers in nuclear medicine and ultrasound microbubbles functionalised with specific targeting agents binding to endothelial cell receptors expressed by the vulnerable plaque- although even dumb bubbles may stick over a damaged endothelium [70,71]. Nowadays, most of tracers aiming at becoming part of an imaging biomarker are based on the radiolabelling of biochemical reporting molecules and laboratory biomarkers and, although dosimetric issues remain a concern for extensive and serial clinical applications, this field represents the most promising approach to molecular and cell imaging. However, non-ionising radiation (NIR) imaging, such as optical imaging [72] and MRS and MRI, is moving forward to overcome their limitations of in-depth penetration and quenching (optical imaging) and sensitivity in MRI and MRS. The latter has made a formidable progress by exploiting hyperpolarised 13C labelled tracers, such as 13C-pyruvate to assess myocardial metabolism [73]. The smart ligand approach can also be used with CMR. Although we can assess and monitor with bioimaging and biochemistry markers stable chronic heart failure, we still miss a reliable marker of acute heart failure and pulmonary congestion, which is different from the hemodynamic congestion currently measured with invasive or non-invasive approach.

Molecular and cellular imaging is a newly emerging field in which the modern tools of molecular and cell biology are being married to state-of-art technology for noninvasive imaging [74]. The approach is ideally suited to assess the efficacy of new treatments which are based on gene and cell transfer, for instance to detect *in vivo* gene-transfer, gene-uptake, and gene delivery. It can also detect early stages of disease, such as endothelial cell activation in early atherosclerosis or myocyte apoptosis in early heart failure [74].

CONCLUSION

Imaging and laboratory biomarkers are an essential support to modern practice of medicine, allowing a better identification, severity titration, staging and follow-up of atherosclerosis and heart failure disease. Yet, no single perfect biomarker exists and there is wide room for optimization and integration between clinical evaluation and biomarker evaluation. In general, a targeted approach tailored on the individual patient should be preferred to a carpet diagnostic bombing, which will lead to an exorbitant multiplier of costs, risks and further inappropriate testing. The magic "info-nano-bio" triangle will certainly help to progressively move towards cellular and molecular images but all technological and methodological innovations should prove their clinical added value in the larger framework of sustainability [75].

REFERENCES

[1]   Challenge and opportunity on the critical path on the new medical products. US Department of Health and Human Services Food and Drug Administration. March 2004

[2]   Vasan RS. Biomarkers of cardiovascular diseases: molecular basis and practical considerations. Circulation 2006; 113: 2335-62

[3]   Federici C, Gianetti J, Andreassi MG. Genomic medicine and thrombotic risk: who, when, how and why? Int J Cardiol. 2006; 106: 3-9.

[4]   Marcovina SM, Crea F, Davignon J, Kaski JC, Koenig W, Landmesser U, *et al.* Biochemical and bioimaging markers for risk assessment and diagnosis in major cardiovascular diseases: a road to integration of complementary diagnostic tools. J Intern Med 2007; 261: 214-34.

[5]   Picano E, Pasanisi E, Brown J, Marwick TH. A gatekeeper for the gatekeeper: inappropriate referrals to stress echocardiography. Am Heart J 2007; 154: 285-90

[6]   Redberg RF. The appropriateness imperative. Am Heart J 2007; 154: 201-2.

[7]   Gibson CM, Pride YB, Buros JL, Lord E, Shui A, Murphy SA, *et al.* TIMI Study Group. Association of impaired thrombolysis in myocardial infarction myocardial perfusion grade with ventricular tachycardia and ventricular fibrillation following fibrinolytic therapy for ST-segment elevation myocardial infarction. J Am Coll Cardiol. 2008; 51: 546-51

[8]   Bonow RO. Is appropriateness apropriate? J Am Coll Cardiol. 2008; 51: 1290-1

[9]   Trisorio Liuzzi MP, Attolini E, Quaranta R, Paparella D, Cappabianca G, Di Serio F, *et al.* Laboratory request appropriateness in emergency: impact on hospital organization. Clin Chem Lab Med. 2006; 44: 760-4.

[10]   Chung ES, Leon AR, Tavazzi L, Sun JP, Nihoyannopoulos P, Merlino J, *et al.* Results of the Predictors of Response to CRT (PROSPECT) trial. Circulation. 2008; 117: 2608-16.

[11]   Sicari R, Nihoyannopoulos P, Evangelista A, Kasprzak J, Lancellotti P, Poldermans D, *et al.* on behalf of the European Association of Echocardiography. Stress echocardiography expert consensus statement: European Association of Echocardiography (EAE) (a registered branch of the ESC). Eur J Echocardiogr 2008; 9: 415-37

[12]   Redberg RF. Cardiovascular risk factors and Medicare costs. N Engl J Med 1999; 340: 813

[13]   Antonielli E, Pizzuti A, Pálinkás A, Tanga M, Gruber N, Michelassi C, *et al.* Clinical value of left atrial appendage flow for prediction of long-term sinus rhythm maintenance in patients with nonvalvular atrial fibrillation. J Am Coll Cardiol. 2002; 39: 1443-9

[14]   Lang RM, Mor-Avi V, Sugeng L, Nieman PS, Sahn DJ. Three-dimensional echocardiography: the benefits of the additional dimension. J Am Coll Cardiol. 2006 21; 48: 2053-69

[15]   Picano E. Stress echocardiography: a historical perspective. Am J Med. 2003; 114: 126-30

[16]   Roman MJ, Naqvi TZ, Gardin JM, Gerhard-Herman M, Jaff M, Mohler E. American Society of Echocardiography; Society of Vascular Medicine and Biology. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. J Am Soc Echocardiogr. 2006; 19: 943-54

[17]   Schroeder S, Achenbach S, Bengel F, Burgstahler C, Cademartiri F, de Feyter P, *et al.* Working Group Nuclear Cardiology and Cardiac CT; European Society of Cardiology; European Council of Nuclear Cardiology. Cardiac computed tomography: indications, applications, limitations, and training requirements: report of a Writing Group deployed by the Working Group Nuclear Cardiology and Cardiac CT of the European Society of Cardiology and the European Council of Nuclear Cardiology. Eur Heart J. 2008; 29: 531-56.

[18]   Chareonthaitawee P, Kaufmann PA, Rimoldi O, Camici PG. Heterogeneity of resting and hyperemic myocardial blood flow in healthy humans. Cardiovasc Res. 2001; 50: 151-61.

[19]   Emdin M, Pompella A, Paolicchi A. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation. 2005; 112: 2078-80.

[20]   Gastaldelli A, Miyazaki Y, Mahankali A, Berria R, Pettiti M, Buzzigoli E, *et al.* The effect of pioglitazone on the liver: role of adiponectin. Diabetes Care. 2006 29: 2275-81.

[21]   Iervasi G, Pingitore A, Landi P, Raciti M, Ripoli A, Scarlattini M, *et al.* Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease. Circulation. 2003; 107: 708-13

[22]   Ferrannini E, Sironi AM, Iozzo P, Gastaldelli A. Intra-abdominal adiposity, abdominal obesity, and cardiometabolic risk. Eur Heart J Suppl 2008; 10: B4-10.

[23]   Ross J Jr. Assessment of ischemic regional myocardial dysfunction and its reversibility. Circulation 1986; 74: 1186-90.

[24]   Thim T, Hagensen MK, Bentzon JF, Falk E. From vulnerable plaque to atherothrombosis. J Intern Med. 2008; 263: 506-16.

[25] Blankenberg FG, Mari C, Strauss HW. Development of radiocontrast agents for vascular imaging: progress to date. Am J Cardiovasc Drugs. 2002; 2: 357-65.

[26] Higuchi T, Bengel FM, Seidl S, Watzlowik P, Kessler H, Hegenloh R, *et al.* Assessment of alphavbeta3 integrin expression after myocardial infarction by positron emission tomography. Cardiovasc Res. 2008; 78: 395-403.

[27] Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, *et al. In vivo* 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J Am Coll Cardiol. 2006; 48: 1818-24.

[28] Ogawa M, Magata Y, Kato T, Hatano K, Ishino S, Mukai T, *et al.* Application of 18F-FDG PET for monitoring the therapeutic effect of antiinflammatory drugs on stabilization of vulnerable atherosclerotic plaques. J Nucl Med. 2006; 47: 1845-50.

[29] Prontera C, Fortunato A, Storti S, Mercuri A, Longombardo G, Zucchelli GC, *et al.* Evaluation of analytical performance of the Siemens ADVIA TnI ultra immunoassay. Clin Chem. 2007; 53: 1722-3

[30] Greenland P, LaBree L, Azen SP, Doherty TM, Detrano RC. Coronary artery calcium score combined with Framingham score for risk prediction in asymptomatic individuals. JAMA 2004; 291: 210-5

[31] Redberg RF, Walsh J. Pay Now, Benefits May Follow -- The Case of Cardiac Computed Tomographic Angiography N Engl J Med. 2008 N; 359: 2309-2311

[32] Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation. 1986; 74: 1399-406

[33] The Royal College of Radiologists. Referral guidelines. Making the best use of clinical radiology services. Sixth edition 2007. London. The Royal College of Radiologists

[34] Serruys PW, García-García HM, Buszman P, Erne P, Verheye S, Aschermann M, *et al.* Integrated Biomarker and Imaging Study-2 Investigators. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. Circulation. 2008; 118: 1172-82

[35] Picano E, Landini L, Distante A, Salvadori M, Lattanzi F, Masini M, *et al.* Angle dependence of ultrasonic backscatter in arterial tissues: a study *in vitro*. Circulation. 1985; 72: 572-6

[36] Kawasaki D, Tsujino T, Fujii K, Masutani M, Ohyanagi M, Masuyama T. Novel use of ultrasound guidance for recanalization of iliac, femoral, and popliteal arteries. Catheter Cardiovasc Interv. 2008; 71: 727-33

[37] Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med. 1987; 316: 1371-5

[38] Li JJ, Zhu CG, Yu B, Liu YX, Yu MY. The role of inflammation in coronary artery calcification. Ageing Res Rev. 2007 ; 6: 263-70.

[39] Neglia D, Rimoldi O, Kaufmann PA, Camici PG. Radionuclide PET and PET/CT in coronary artery disease. Curr Pharm Des. 2008; 14: 1798-814.

[40] Einstein AJ, Henzlova MJ, Rajagopalan S. Estimating risk of cancer associated with radiation exposure from 64-slice computed tomography coronary angiography. JAMA. 2007; 298: 317-23.

[41] Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. N Engl J Med. 2007; 357: 2277-84

[42] Amis ES Jr, Butler PF, Applegate KE, Birnbaum SB, Brateman LF, Hevezi JM, *et al.* American College of Radiology. American College of Radiology white paper on radiation dose in medicine. J Am Coll Radiol. 2007; 4: 272-84.

[43] Pichler BJ, Judenhofer MS, Pfannenberg C Multimodal imaging approaches: PET/CT and PET/MRI. Handb Exp Pharmacol. 2008; 185: 109-32.

[44] American College of Cardiology; American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). J Am Coll Cardiol 2005 20; 46: e1-82.

[45] Rodríguez E, Soler R. New MR insights of cardiomyopathy. Eur J Radiol. 2008 ; 67: 392-400

[46] Milting H, Ellinghaus P, Seewald M, Cakar H, Bohms B, Kassner A, *et al.* J Plasma biomarkers of myocardial fibrosis and remodeling in terminal heart failure patients supported by mechanical circulatory support devices. Heart Lung Transplant. 2008; 27: 589-96.

[47] Clerico A, Recchia FA, Passino C, Emdin M. Cardiac endocrine function is an essential component of the homeostatic regulation network: physiological and clinical implications. Am J Physiol Heart Circ Physiol. 2006 Jan; 290(1): H17-29

[48] Daniels LB, Maisel AS. Natriuretic peptides. J Am Coll Cardiol. 2007; 50: 2357-68.

[49] Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, *et al.* American Heart Association; Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; Council on Epidemiology and Prevention. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation 2006; 113: 1807-16.

[50] Robin NH, Tabereaux PB, Benza R, Korf BR. Genetic testing in cardiovascular disease. J Am Coll Cardiol. 2007; 50: 727-37.

[51] van Spaendonck-Zwarts KY, van den Berg MP, van Tintelen JP., DNA analysis in inherited cardiomyopathies: current status and clinical relevance. Pacing Clin Electrophysiol. 2008; 31: S46-9.

[52] Colombo MG, Botto N., Vittorini S., Paradossi U, Andreassi MG**.** Clinical Utility of Genetic Tests for Inherited Hypertrophic and Dilated Cardiomyopathies Journal Cardiovascular Ultrasound 2008

[53] Pasotti M, Klersy C, Pilotto A, Marziliano N, Rapezzi C, Serio A, *et al.* Long-term outcome and risk stratification in dilated cardiolaminopathies. J Am Coll Cardiol 2008, 52: 1250-60.

[54] Franchini M, Peyvandi F, Mannucci PM. The genetic basis of coronary artery disease: from candidate genes to whole genome analysis. Trends Cardiovasc Med. 2008; 18: 157-62.

[55] La Rosée K, Huntgeburth M, Rosenkranz S, Böhm M, Schnabel P. The Arg389Gly beta1-adrenoceptor gene polymorphism determines contractile response to catecholamines. Pharmacogenetics. 2004; 14: 711-6.

[56] Grünig E, Janssen B, Mereles D, Barth U, Borst MM, Vogt IR, *et al.* Abnormal pulmonary artery pressure response in asymptomatic carriers of primary pulmonary hypertension gene. Circulation. 2000 Sep 5; 102(10): 1145-50.

[57] Pennell DJ, Sechtem UP, Higgins CB, Manning WJ, Pohost GM, Rademakers FE, *et al.* Society for Cardiovascular Magnetic Resonance; Working Group on Cardiovascular Magnetic Resonance of the European Society of Cardiology. Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. Eur Heart J. 2004; 25: 1940-65

[58] Picano E. Informed consent and communication of risk from radiological and nuclear medicine examinations: how to escape from a communication inferno. BMJ. 2004; 329: 849-51

[59] Dokainish H, Abbey H, Gin K, Ramanathan K, Lee PK, Jue J Usefulness of tissue Doppler imaging in the diagnosis and prognosis of acute right ventricular infarction with inferior wall acute left ventricular infarction. Am J Cardiol. 2005; 95: 1039-42

[60] Mueller C, Laule-Kilian K, Schindler C, Klima T, Frana B, Rodriguez D, *et al.* Cost-effectiveness of B-type natriuretic peptide testing in patients with acute dyspnea. Arch Intern Med. 2006; 166: 1081-7

[61] Picano E, Frassi F, Agricola E, Gligorova S, Gargani L, Mottola G. Ultrasound lung comets: a clinically useful sign of extravascular lung water. J Am Soc Echocardiogr. 2006; 19: 356-63

[62] Gargani L, Frassi F, Soldati G, Tesorio P, Gheorghiade M, Picano E. Ultrasound lung comets for the differential diagnosis of acute cardiogenic dyspnoea: a comparison with natriuretic peptides. Eur J Heart Fail. 2008; 10: 70-7.

[63] Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C *et al.* Multiple biomarkers for the prediction of first major cardiovascular events and death. The New England journal of medicine 2006; 355: 2631-2639.

[64] Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, *et al.* Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. Arteriosclerosis, thrombosis, and vascular biology 2007; 27: 127-133.

[65]   Gerszten RE, Wang TJ. The search for new cardiovascular bio-markers. Nature 2008; 451: 949-952.

[66]   Ross R. Atherosclerosis — An Inflammatory Disease. N Engl J; Med1999; 340: 115

[67]   Ridker PM. Inflammatory biomarkers and risks of myocardial infarction, stroke, diabetes, and total mortality: implications for longevity. Nutr Rev. 2007; 65: S253-9.

[68]   Jaffe AS. Key issues in the developing synergism between cardio-vascular imaging and biomarkers. Clin Chem. 2008; 54: 1432-42.

[69]   McGeechan K, Macaskill P, Irwig L, Liew G, Wong TY. Assessing new biomarkers and predictive models for use in clinical practice: a clinician's guide. Arch Intern Med 2008; 168: 2304-10

[70]   Venneri L, Poggianti E, Jambrik Z, Varga A, Palinkas A, Picano E. The elusive prognostic value of systemic endothelial function in patients with chest pain syndrome. Int J Cardiol. 2007; 119: 109-11

[71]   Tsutsui JM, Maciel RR, Costa JM, Andrade JL, Ramires JF, Mathias W Jr. Hand-carried ultrasound performed at bedside in cardiology inpatient setting - a comparative study with comprehen-sive echocardiography. Cardiovasc Ultrasound. 2004; 2: 24

[72]   Tanaka A, Imanishi T, Kitabata H, Kubo T, Takarada S, Tanimoto T, *et al.* Morphology of Exertion-Triggered Plaque Rupture in Pa-tients With Acute Coronary Syndrome: An Optical Coherence To-mography Study. Circulation, Dec 2008; 118: 2368 - 2373.

[73]   Schroeder MA, Cochlin L, Heather L, Clarke K, Radda G, Tyler D. *In vivo* assessment of pyruvate dehydrogenase flux in the heart us-ing hyperpolarized carbon-13 magnetic resonance PNAS 2008; 105: 12051–6.

[74]   Pennell DJ, Sechtem UP, Higgins CB, Manning WJ, Pohost GM, Rademakers FE, *et al.* Society for Cardiovascular Magnetic Reso-nance; Working Group on Cardiovascular Magnetic Resonance of the European Society of Cardiology. Clinical indications for car-diovascular magnetic resonance (CMR): Consensus Panel report. Eur Heart J 2004; 25: 1940-65

[75]   Picano E. Sustainability of medical imaging. BMJ. 2004; 328: 578-80.