Perspective
Future Cardiology

Multimarker approach for identifying and documenting mitigation of cardiovascular risk

Marc S Penn*[1,2] & Andrea B Klemes[3]
[1]Cleveland Heart Lab, 6701 Carnegie Avenue, Cleveland, OH 44106, USA
[2]Summa Cardiovascular Institute, Summa Health System, 525 East Market Street, Akron, OH 44309, USA
[3]MDVIP, Inc., 1875 Corporate Boulevard, Northwest Boca Raton, FL 33431, USA
*Author for correspondence: Tel.: +1 216 835 8503 ■ Fax: +1 866 299 7071 ■ marc.s.penn@gmail.com

The widespread use of lipids to define risk has been a success based on the dramatic decrease in the incidence of transmural myocardial infarctions. This success and the fact that many patients with normal lipid levels go on to have acute coronary syndrome have led to investigations on the use of nonlipid-based inflammatory biomarkers to predict risk. Interestingly, as the physiology reflected by distinct biomarkers is better understood, there is increasing interest in multimarker approaches to determine risk and where a given patient may be on a spectrum of risk. In this perspective, we review data from over 95,000 patients who had a multimarker annual wellness panel to demonstrate the utility of multiple markers in defining those patients at risk. We discuss a novel multimarker panel for cardiovascular risk, define the differences between a multimarker approach and expensive amalgamations of multiple markers, and discuss how the field may develop in the future.

The growing understanding of the molecular pathways that regulate the development of acute and chronic disease states has led to the launch of a myriad of biomarkers, which claim to risk-stratify patients for the development of different conditions. The presumption with this type of strategy is that with modification of preventive strategies, we can prevent the development of acute illness and the chronic manifestations that follow.

One can argue that, by all measures, the strategy works if the biomarker is pathologically linked to the underlying disease. Two excellent examples are fecal occult blood testing or colonoscopy for the detection of polyps in patients at risk for colon cancer and hypercholesterolemia in patients at risk for myocardial infarction. The aggressive use of screening for precancerous and cancerous lesions of the colon has led to a reduction in the death rate of colon cancer [1] and the aggressive use of lipid panels and cholesterol-lowering agents has decreased the incidence of ST elevation myocardial infarctions by over 36% from 1997 to 2005 [2].

As the field moved forward and ventured into more complex chronic disease states, the link between pathophysiology and the disease of a single biomarker has faltered. Rather, biomarkers reflect the physiological state rather than pathophysiology, regardless of whether they are mechanistically involved in disease progression [3]. This has led many to propose that multiple biomarkers are required to more fully stratify patient risk for chronic complex disease states [4]. At the same time, while biomarker risk stratification has become more complex, many have argued that the approach is overimplemented and that healthy patients are exposed to costly testing.

The goal of this article is to report the results of 95,144 patients who underwent biomarker screening to assess their state of wellness. The biomarker screening panel was developed between a collaboration of the physician leadership of MD Value In Prevention Inc. (MDVIP, FL, USA) and Cleveland Heart Lab, Inc. (OH, USA). Importantly, patients in the MDVIP personalized wellness model pay an annual fee for an annual wellness program, which includes the wellness screen; thus, these patients have sufficient interest in their health to pay out of pocket for this type of care. Many would characterize this population as 'the worried well' and is the very population that, because of their engagement in their own health, many would argue risk stratification is unnecessary. We will then discuss a broader multimarker approach for the stratification of cardiovascular risk and conclude with the current and future role for multimarker testing for cardiovascular risk.

Multimarker screening for wellness

Screening for a patient's state of wellness can take on many forms. The goal of any screening test is to be easy to implement and have a high sensitivity and a low relative cost. The goal of

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■ cardiovascular risk
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the MDVIP annual wellness panel is to broadly assess risk factors of cardiovascular disease, since cardiovascular disease remains the number one killer of Americans and guideline-based age-appropriate cancer screening is covered by Medicare and most commercial insurance.

The wellness screening panel is outlined in Box 1. It focuses on known risk factors for cardiovascular disease including diabetes, hypercholesterolemia, apoB, apoA1 and myeloperoxidase, a measure of vulnerable plaque [5]. The panel also included a complete blood count and basic metabolic panel (Na^+, K^+, Cl^-, $HCO3^-$, blood urea nitrogen and creatinine). The presence of hypertension was assessed during the physical examination, as was BMI. It should be noted that the decision matrix applied to the development of this wellness screening panel was complex. We do not represent that this is an ideal wellness screening panel for all groups and acknowledge that the analyses that follow are limited, based on what could and could not be included.

Between 1 January and 31 December 2011, 95,144 patients underwent wellness panel screening by their MDVIP physician. All analyses were carried out on de-identified data; therefore, specifics about the patients with regard to past medical history, physical examination and medication profile were not available. Figure 1A depicts the age distribution of the population tested.

Figure 1B shows the percentage of patients that were positive for each marker. A data set of 95,144 patients allows us to investigate the relative discriminatory power of each parameter, as well as whether there is a high degree of correlation between parameters. We observed that a large percentage of patients had an abnormal HDL2 (~40%) and we also observed through further analysis that HDL3 was highly correlated with apoA1 (R^2 = 0.97). We did not observe a high degree of correlation between other markers. Based on the relative lack of discrimination of HDL2 and the high correlation of HDL3 and apoA1, the analysis below is based on all of the remaining markers [6].

Of the markers in the annual wellness panel, the only one that is reimbursed for screening is the lipid panel. Based on a cut-off of 130 mg/dl, 27% of patients were deemed at risk for cardiovascular disease. If the cut-off were lowered to the more aggressive 100 mg/dl, then the number of patients at risk would rise to 33%. A total of 30% of patients had no positive markers in the annual wellness panel. This suggests that the multimarker annual wellness panel identified 70% of patients with some risk, 37–43% more patients than would have been identified with LDL alone.

Figure 1C shows that most patients who demonstrated some level of risk had multiple positive markers (41.1%); however, a significant number of patients (28.9%) had only a single elevated risk marker. Figure 1D depicts the percentage of patients who had a single positive marker, stratified by the marker that was positive.

This real-life example demonstrates the utility of a multimarker approach to screen for evidence of cardiovascular risk. Clearly, no one marker would have stratified this patient cohort appropriately and the early identification of patients at risk with this annual wellness panel allows the patient to address lifestyle issues related to risk, and their treating physician to address medication and lifestyle changes, which could prevent or treat chronic disease states with the patient. From a risk standpoint, as could be the case for self-insured employers, such an approach allows for a deeper understanding of the risk associated with a cohort of people compared with any single risk marker.

Beyond just identifying patients at risk, a multimarker approach allows for the possibility to stratify a given patient's risk. For example, multiple studies have shown that knowledge of both the inflammatory state and lipid status offers additive information for risk [7,8]. With the multimarker annual wellness panel, we were able to define patients at increased risk based on high LDL levels or high myeloperoxidase (MPO), and patients at a greater risk with both

Box 1. Cardiovascular risk markers in the MDVIP wellness panel by the Cleveland Heart Lab.

Diabetes
- HgA1C (<5.7%)
- Fasting glucose (<100 mg/dl)

Lipids (mg/dl)
- LDL (<130)
- HDL (M: >45; F: >50)
- Triglyceride (<150)
- HDL2 (M: >10 F: >15)
- HDL3 (M: >30; F: >25)
- apoB (<109)
- apoA1 (M: >118; F: >145)

Vascular health (pmol/ml)
- MPO (<480)

Normative values are listed in parentheses.
F: Female; M: Male; MPO: Myeloperoxidase.

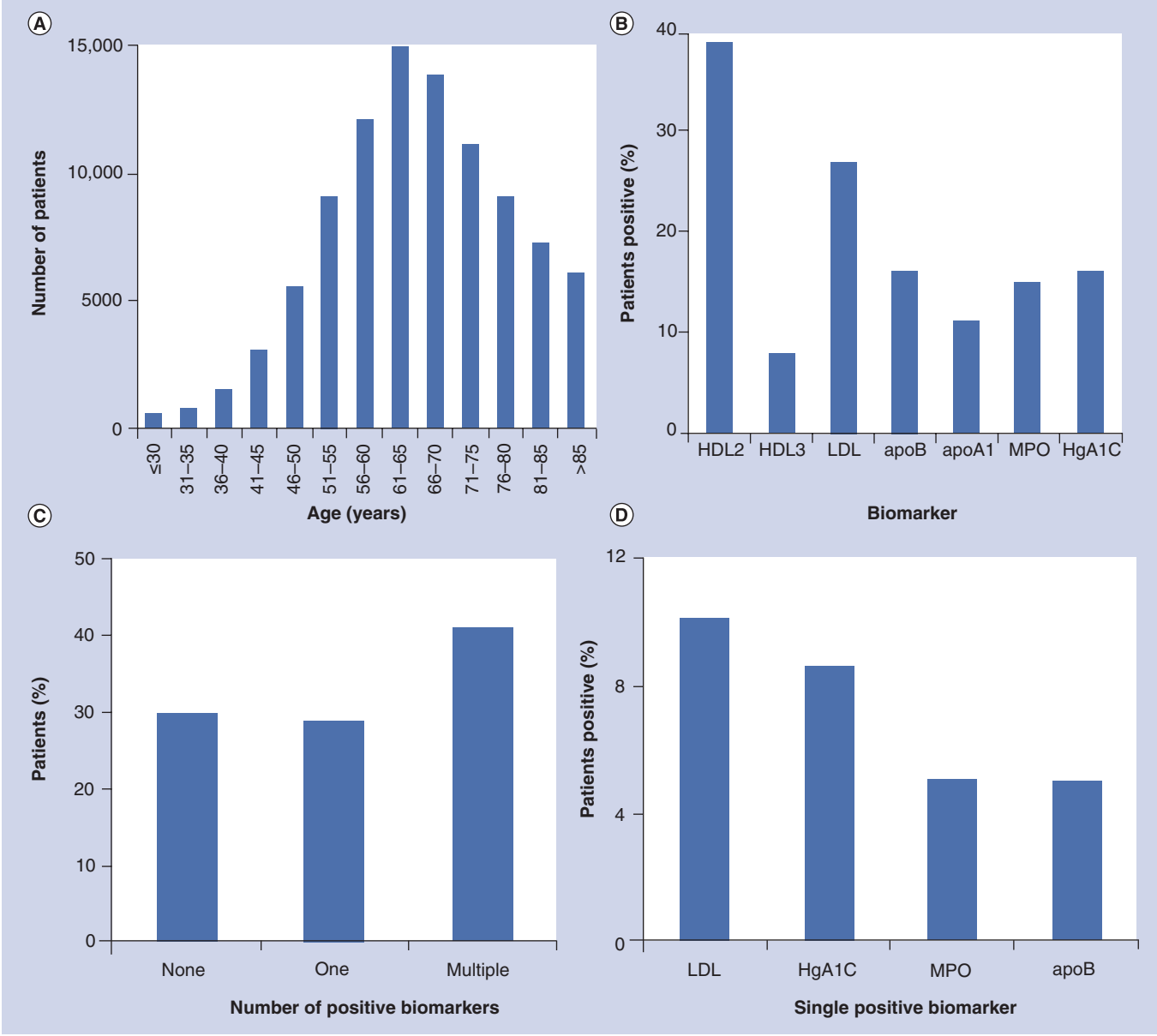

Figure 1. Summary of MDVIP data from 95,144 patients. (A) Age distribution of patient cohort; **(B)** percentage of patients positive for the indicated biomarker; **(C)** percentage of patients with completely normal biomarker panel, a single abnormal marker or multiple abnormal biomarkers; **(D)** percentage of patients with only the indicated biomarker positive.
MPO: Myeloperoxidase.

a high LDL and MPO (Figure 2). Such information can aid physicians in determining the acuity of risk and whether just diet and exercise, or diet, exercise and pharmacological intervention are appropriate.

Multimarker approach to assess acuity of risk

The response-to-injury hypothesis of atherosclerosis proposes that, following initiation of the atherosclerotic process due to arterial injury, inflammation occurs and propagates the disease. As discussed above, the screening and treatment of hypercholesterolemia has significantly impacted the prevalence of the disease process to the point that at least 50% of the patients who present with acute myocardial infarction have 'normal' cholesterol levels due to treatment or lifestyle modification [9]. Therefore, at the current time, the measurement of lipid levels alone is insufficient to determine whether patients are at risk of a cardiovascular event.

Ridker and colleagues have worked for over a decade to enhance our understanding of the utility of high-sensitivity CRP (hsCRP) for risk stratification of patients at risk of coronary

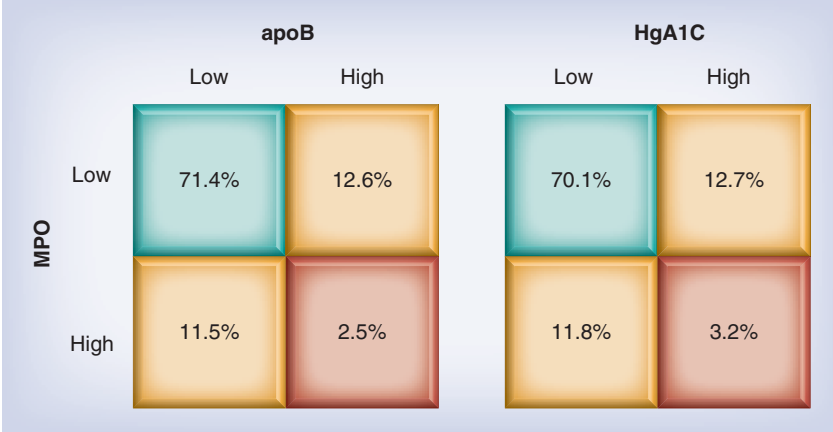

Figure 2. Demonstrations of how combining myeloperoxidase with apoB or HgA1C reclassify patients from increased risk (yellow) to high risk (red).
MPO: Myeloperoxidase.

events [7,8,10]. hsCRP stratifies the normal range for the classic CRP test. Their numerous studies unequivocally demonstrate that elevated hsCRP levels identify patients at risk and that there is additive utility in defining a patient's lipid status and inflammatory status [7]. The work on hsCRP highlights the fact that markers of inflammation that can be elevated in multiple disease states have value in the assessment of cardiovascular risk when applied to the appropriate clinical populations, when the reference range that reflects cardiovascular risk is defined and when the physiology being represented is understood.

Based on the sum total of the data on lipid and cardiovascular risk markers, we have proposed that the complex space of cardiovascular risk stratification can be divided into three general groups of risk acuity – lifelong (classic lipid panel), mid-term (decades, advanced lipid testing) and near-term (years, inflammatory markers; Figure 3).

One advantage of a multimarker approach to

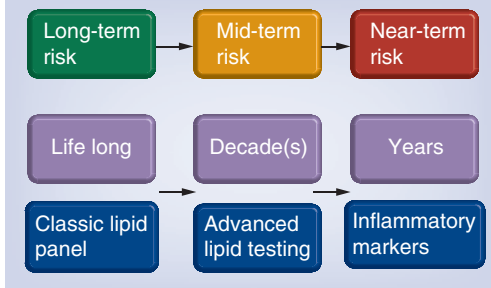

Figure 3. Proposed strategy for classifying different classes of cardiovascular risk factors based on temporal relationship with risk.
Figure courtesy of Cleveland Heart Lab, Inc. (OH, USA).

risk stratification is the concept that if the physiology reflected by the marker is well understood, then not only risk, but the acuity of risk, can be assessed. This continuum of risk based on oxidation/inflammation markers is based on the fact that specific markers of inflammation have shown nearer-term evidence of cardiovascular events in specific populations, such as chest pain patients with MPO [5], while others have demonstrated longer-term risk [7,11]. Furthermore, populations selected based on increased inflammatory markers for statin trials (JUPITER trial [9]) have led to a rapid demonstration of a survival advantage compared with statin trials in which patients were enrolled based on elevated lipids or secondary prevention [12].

Based on this concept, we have developed a multimarker approach that does not simply define whether a patient is at risk, but the potential acuity of his or her risk (Figure 4). This six-marker panel based on oxidation and inflammation assesses the patient for evidence of lifestyle risk through to the presence of vulnerable plaque.The markers on this panel from lowest to highest acuity of risk are:

- F2-isoprostane (F2-iso: a general marker of systemic oxidation generated from free radical-mediated oxidation of arachidonic acid. High levels of F2-iso portend long-term cardiovascular [13] and cancer risk [14]. Strategies to lower F2-iso include quitting smoking and conditioning;
- Oxidized LDL (oxLDL): a marker of apoB oxidation/modification. A high level of oxLDL greatly increases the risk of the patient developing metabolic syndrome in the ensuing 5 years [15]. Increased oxLDL in patients with known coronary artery disease suggests increased risk of major adverse coronary events. One can lower oxLDL through diet, weight loss, lowering of cholesterol levels and control of blood pressure;
- Microalbuminuria: a marker of endothelial dysfunction. Increased levels of albumin in the urine are correlated with increasing risk of cardiovascular events. This increase in risk is independent of whether the patient had diabetes or not [16]. Strategies to lower microalbuminuria include angiotensin-converting enzyme inhibitors and control of blood pressure;
- hsCRP: a general marker of inflammation, which, at low levels, represents the degree of vasculopathy. An increase in hsCRP marks an increased risk of cardiovascular events and a

decrease in event rates if these patients are treated with aspirin [11] and/or statin therapy [9]. hsCRP can be lowered by weight loss, statin therapy and antiplatelet therapy. Importantly, hsCRP has not been shown to be associated with vulnerable plaque [5], suggesting it may represent plaque burden more than plaque activity.

- Lp-PLA2: Lp-PLA2 is elevated in the bloodstream in response to macrophage activation in the intima–media of atherosclerotic plaque. Macrophage activation under the cap of the plaque is associated with vulnerable plaque formation [17]. Increased levels of Lp-PLA2 reflect increased risk for major adverse cardiac events including myocardial infarction and stroke. Lp-PLA2 can be decreased through measures that restore vascular health, including statin therapy, antiplatelet therapy and controlling postprandial blood sugar levels. Increased levels mark a patient at near-term risk;
- MPO is released by neutrophils and monocytes at sites of inflammation. The MPO assay quantifies the amount of free MPO in the bloodstream. Free MPO in the bloodstream is increased in response to vascular inflammation, including vulnerable plaque formation, fissures and erosions in the luminal surface of the atherosclerotic plaque [5, 18]. Similar to Lp-PLA2, MPO can be decreased through measures that restore vascular health and increased levels mark a patient at near-term risk.

Perhaps one of the stronger demonstrations for the utility of a multimarker approach is based on data from the higher acuity risk markers in the panel described above. Based on our understanding of the physiology reflected by Lp-PLA2 and MPO, increases in either of these biomarkers indicates the presence of vulnerable plaque and increased risk for acute coronary syndrome [5,17]. This is true in patients with and without a prior history of coronary artery disease [5,19]. The difference between the two markers is that Lp-PLA2 tracking risk of adverse cardiac events due to flammation within the intima–media of the atherosclerotic lesion and MPO tracks risk associated h the luminal aspect of the lesion. If, in fact, -PLA2 and MPO define distinct physiologies plaque vulnerability, as described above, plaque oture would be characterized by an elevated -PLA2 and MPO. Furthermore, Lp-PLA2 and PO would not identify the same clinical populons. To test this hypothesis, we compared the relation between high Lp-PLA2 and MPO in 00 patients from a preventive cardiology and an executive health clinic. Figure 5 reveals that, in such a population, approximately 6% of patients have increased risk due to luminal irregularities, while approximately 5% of patients are at risk based on inflammation within the intima–media under the collagen cap. Interestingly, in this asymptomatic

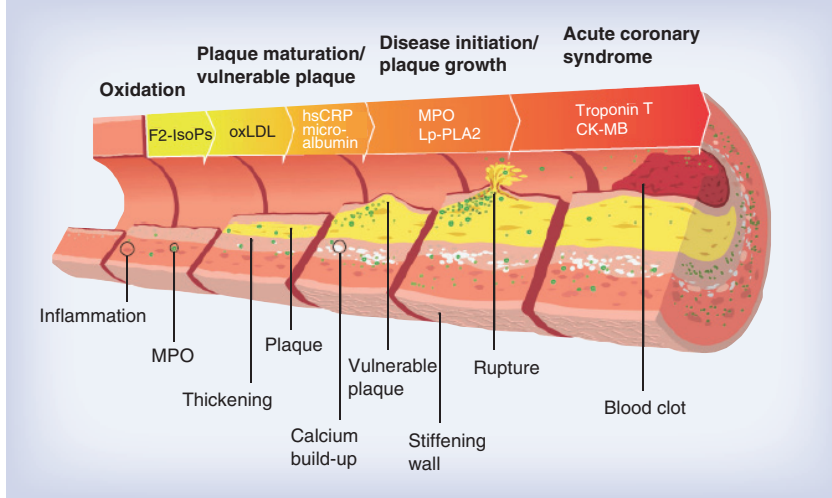

Figure 4. Multimarker approach for identifying patients at risk and assessing the degree of risk.
CK-MB: Creatinine kinase isoform MB; hsCRP: High-sensitivity CRP; MPO: Myeloperoxidase; oxLDL: Oxidized LDL.
Figure courtesy of Cleveland Heart Lab, Inc. (OH, USA).

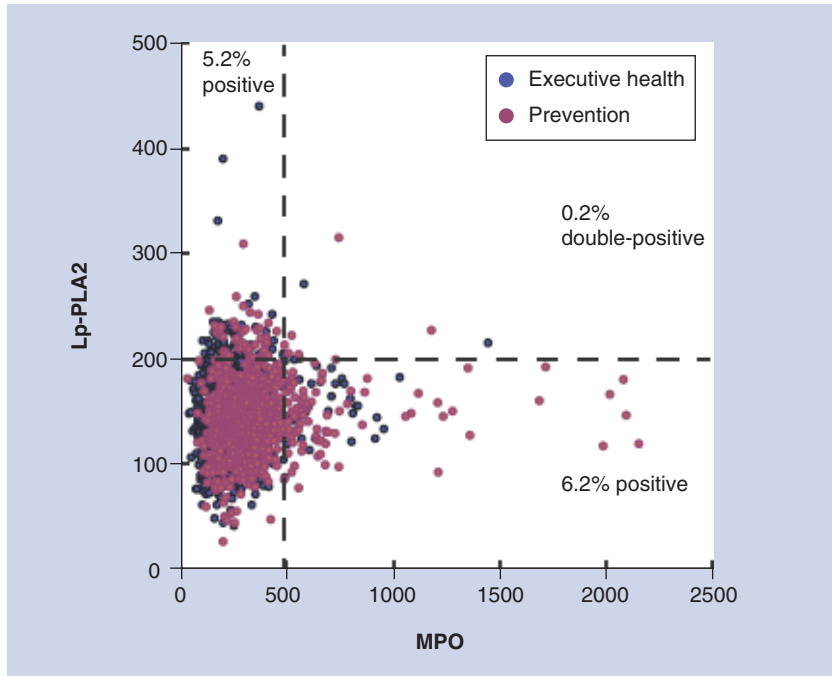

Figure 5. Risk based on vulnerable plaque risk markers in a stable clinical population of patients. Patients originate from Preventive Cardiology (red) or Executive Health (blue) practices. MPO and Lp-PLA2 levels measured in 2761 individual patients. 6.2 and 5.2% of patients had elevated MPO or Lp-PLA2 levels, respectively. Of the 2761 patients, only six (<0.2%) had both elevated MPO and Lp-PLA2 levels.
MPO: Myeloperoxidase.
Figure courtesy of Cleveland Heart Lab, Inc. (OH, USA).

p-PLA2在动脉粥样硬化斑块中随着巨噬细胞在内膜-介质中的激活而升高

根据我们对Lp-PLA2和MPO所反映的生理学的理解，这些生物标志物的增加都表明了易损斑块的存在和急性冠状动脉综合征的风险的增加

这两种标记物的不同之处在于，Lp-PLA2追踪由动脉粥样硬化病变的内膜-中膜内炎症引起的不良心脏事件的风险，而MPO追踪与病变的管腔方面相关的风险。

outpatient population, approximately 0.2% of patients have both markers elevated. These data demonstrate two important concepts regarding multimarker approaches. First, if one understands the physiology reflected in different markers, one can begin to combine them to further stratify risk; second, in the case of MPO and Lp-PLA2, if one were to take a single marker approach, this would suggest that one is comfortable with assessing only one side of the coin or only half of the patient's potential for risk.

Multiple studies have demonstrated that combining multiple markers allows for further risk stratification. Analyses of the PROVE-IT trial [8] and the Women's Health study [7] have both demonstrated that low cholesterol and hsCRP indicates low risk, that either marker elevated indicates elevated risk and that both markers elevated indicates yet greater risk than either marker alone. Similarly, Heslop and colleagues demonstrated that patients who underwent left elective angiography had a 5.33-fold increased hazard ratio for mortality if either MPO or hsCRP were elevated, but they had a further 4.33-fold increase in hazard ratio if both MPO and hsCRP were elevated [20].

It is important to note that a multimarker approach does not only include blood-borne biomarkers. There are multiple studies that show the utility of combining imaging with inflammatory biomarkers. One way to conceptualize a combined imaging and blood biomarker approach is that imaging captures the anatomical risk and the inflammatory biomarkers capture the biological risk of an event [21,22]. Furthermore, it is more convenient, cost-effective and, in some cases, safer (i.e., coronary calcium score or coronary angiography) to repeat blood testing than to repeat an imaging test; therefore, one can follow blood-borne biomarkers in the near-term to determine whether treatment strategies are having an impact at a shorter time interval than imaging.

Multimarker testing for cardiovascular risk

We have described two ongoing multimarker approaches for stratifying cardiovascular risk:

- The >90,000 patient cohort demonstrates that limiting risk screening to lipid abnormalities significantly inhibits our ability to identify patients at risk. These findings are consistent with the current reality that approximately 50% of patients who have acute coronary syndrome have controlled lipoprotein levels;
- The oxidation/inflammation multimarker approach developed by Cleveland Heart Lab, Inc. allows not only for identification of patients at risk, but also determines the acuity of their risk. Such an approach identifies those patients best suited for primary lifestyle modification and those who warrant early pharmacologic intervention.

Our growing understanding of the physiology characterized by individual biomarkers has led many to propose and demonstrate the additive utility and synergies associated with specific combinations of biomarkers. The data herein supports the concept. The question becomes what constitutes a multimarker approach and where can a multimarker approach offer value?

What constitutes a multimarker approach?

With respect to what constitutes a multimarker approach for cardiovascular risk assessment, it is our opinion that this should not constitute a listing of several to dozens of markers that individually may offer insight into a patient's risk, but collectively do not offer additive or synergistic information, as we have outlined based in the panel above. All too often, physicians are encouraged to order a multitude of tests based on 'more is better', with little evidence of any additive value of the combination of the tests. Unfortunately, at times, the costs for this approach can run into several hundred or even thousands of dollars, driving up costs with little scientific or medical evidence of benefit. It is this indiscriminate use of multiple markers that we believe has turned many against a multimarker approach and has largely led the field 'to throw the baby out with the bath water'. Hopefully, as we have demonstrated with the MDVIP annual wellness panel and the Cleveland Heart Lab, Inc. inflammation cardiovascular risk panel, carefully thought out, scientifically and medically based panels can offer value at a reasonable price. While for contractual and proprietary reasons we cannot reveal the costs of these panels, we can share that they cost significantly less than a handful of hundreds of dollars.

Where can a multimarker approach add value?

The ability to identify and stratify risk can add value in multiple settings.

Traditional patient–physician interaction

The most obvious setting where a multimarker approach may offer value is on an individual basis

between the physician and the patient. Identifying patients at risk and where they exist on the spectrum of risk allows for individualization of preventive strategy to a greater extent than that which exists in the binary outcome of a single test. Identification of patients with significant free radical generation or lipoprotein oxidation identifies patients who, on balance, have lifestyle risks that can be addressed through behavior modification, ideally preventing the onset of disease and the need for pharmacologic intervention. Identifying and enriching for a patient population that has primarily lifestyle risk can lead to wellness strategies being optimized without the risk of including patients with advanced disease that will not respond to lifestyle changes alone. Furthermore, identification of lifestyle risk via an objective measure allows for measurement of the changes in the objective marker in response to lifestyle modification. Such patient-specific feedback may serve to motivate patients and enhance compliance. Conversely, identification of a patient who has evidence of vulnerable plaque identifies a patient who not only requires institution of pharmacological intervention, but also evaluation for risk factors that may not be obvious, including insulin insensitivity or drivers of chronic inflammation such as gum disease. Unfortunately, physician time, patient interest and costs do not allow for full testing of every patient.

Self-insured employer risk assessment

On a population basis, using a multimarker risk assessment strategy allows the self-insured employer to understand the degree of risk inherent in the covered population to a greater degree than a single-marker approach. There are several potential benefits of risk stratification along a spectrum of risk markers including:

- Allowing deployment of resources in a more efficient and strategic manner. Lifestyle programs and incentives can be targeted at patients whose risk is centered on lifestyle and whose compliance in wellness programs can prevent disease;
- Identifying patients at highest risk for whom aggressive testing and risk-factor modification might offer the greatest return. For example, based on Figure 5, approximately 11% of patients are at increased risk with evidence of vulnerable plaque based on an elevated Lp-PLA2 or MPO; however, one can further refine this population to a smaller cohort with the greatest risk based on an elevated Lp-PLA2 and MPO. Improved outcomes and avoidance of acute events in this refined population has the potential to offer sufficient avoidance of cost-to-pay for the whole risk stratification program for the entire population;
- Quantification of the benefits of a program-wide risk-factor modification. One of the goals of implementing wellness programs within a covered population is assessing the benefits to the general health of the population. The ultimate measure of benefit is a decrease in acute clinical event; unfortunately, it can take a number of years to see a decrease in events. Furthermore, whether the number of events is a true decrease can only be assessed if the baseline risk of the population is defined. Thus, two data points are needed to determine the success of a wellness program: the number of patients at risk at baseline and a measure of how that risk has changed over time. A multimarker approach for cardiovascular risk assessment offers a greater degree of granularity on the risk of a given population than any single marker can. As seen with the MDVIP data set, focusing cardiovascular risk assessment solely on lipid panels leads to poor characterization of cardiovascular risk. Similarly, multimarker assessment allows for better quantification of the benefits associated with a wellness program sooner than a decrease in event rate.

Step-wise risk factor assessment strategy

It should be noted that MDVIP data and its approach highlights a risk-factor assessment strategy that allows for a detailed assessment of patients at risk over time. This approach fits the workflow of concierge and primary care practices where there is sufficient time and physician access so that the information and findings of the multimarker approach can be fully explained to the patient and appropriate next steps can be taken. For example, with the MDVIP panel, if a given patient was found to have increased risk based on the lipid panel, but a normal MPO, the patient could be followed up with a Lp-PLA2 test to further assess for the presence of vulnerable plaque. Similarly, if a patient was found to have a normal lipid panel and a normal MPO, there is no indication for a Lp-PLA2 test, since there is no clear concern for underlying risk of atherosclerosis. However, further assessment with a hsCRP test would be appropriate to further assess atherosclerotic risk. In addition, a normal lipid panel and an abnormal MPO suggests that the next step could be a coronary calcium score or

carotid intimal–medial thickness measurement to determine whether there is evidence of precocious atherosclerosis despite normal lipid levels. Further assessment with oxLDL or hsCRP tests would be appropriate, as well as to further refine risk and define baseline values to be followed during treatment.

Conclusion

Our growing understanding of the molecular pathways involved in the development of acute and chronic disease has led to the discovery of a number of biomarkers associated with the presence and progression of a variety of disease states. This has led many to propose that we can better refine a given patient's risk by measuring multiple biomarkers rather than a single one. As discussed above, there are several characteristics of a multimarker approach that could add value:

- The physiology reflected by the biomarkers is understood and there is an understanding of how the group of biomarkers is additive or synergistic compared with a single measure;
- The multimarker approach is not simply a list of unrelated or redundant markers leading to an expensive panel, in which the combination, of biomarkers does not further refine a patient's risk profile;
- The multimarker approach reflects the disease state such that successful prevention or treatment leads to changes in the overall measured risk profile of the patient.

Unfortunately, in cardiovascular risk assessment, there appears to be a bias in the academic sector against novel biomarkers, and perhaps an even greater bias against a multimarker approach. The most common criticism is the degree of benefit seen over Framingham risk. Ridker and colleagues took this criticism head on over a number of years, culminating in the demonstration of a statin treatment benefit in patients with an elevated hsCRP in the randomized placebo-controlled JUPITER trial. While a great achievement, it needs to be understood that the JUPITER trial was designed and funded to support the development of a pharmaceutical drug and that the revenues associated with biomarkers are such that robust randomized controlled clinical trials cannot be funded by diagnostic companies. Collaborations such as the one described here for the first time between MDVIP and Cleveland Heart Lab, Inc. should serve as a model for how the field can bring forward real-world data to prove or disprove the utility of a multimarker approach for cardiovascular risk assessment.

Future perspective

From the beginning of medical school training, physicians are taught to identify patients' phenotype. Patients' weight, nicotine-stained fingers and teeth, ear creases, webbed feet, yellow eyes and tongue, clubbed fingers, ketotic breath and xanthomas, among others. Neither of us recalls a mentor who said just look at one. We were always taught that it was the constellation of phenotypes that led to diagnoses or, more importantly, directed us in an appropriate direction for further examination, imaging or testing. Years ago, the early stages of laboratory

Executive summary

Background

- Early detection of disease allows the potential for prevention of morbidity and mortality.
- Success in modulating risk from one pathway often increases the need and utility of risk stratification by other pathways.

Multimarker screening for wellness

- Molecular mechanisms of ischemic cardiovascular events are being better defined.
- Beyond lipids, inflammation has a key role in inducing plaque rupture.
- No single marker fully defines a patients' cardiovascular risk profile.

Multimarker approach to assess acuity of risk

- A panel of multiple markers that are not synergistic or additive increases cost unnecessarily without improving risk prediction.
- Understanding the physiology reflected by a specific biomarker allows multiple markers to be used in parallel to more fully define risk without redundancy.

Multimarker testing for cardiovascular risk

- Using a multimarker approach to identify patients prior to a clinical event has the potential to decrease costs if the event can be prevented.
- A multimarker approach has the potential to risk stratify and define risk in self-insured populations to a greater extent than is now possible.

medicine and our understanding of the mechanisms of disease single markers were accepted and made a difference. The impact measuring LDL cholesterol has had on atherosclerosis, as discussed above, is an excellent example. But just as phenotyping patients requires looking at multiple physical characteristics, the growth in our understanding of the mechanisms and biological manifestations of chronic disease have led and will lead to the development of multiple potential biomarkers for the risk stratification of ischemic cardiovascular disease. These biomarkers will take the form of proteins, miRNAs, genotypes and possibly biopsies, and they will be enhanced by imaging techniques that will define the anatomical manifestations of the disease, and perhaps the biology as well [23]. Importantly, these multimarker panels will need to be biologically rational, medically meaningful and modifiable. Their use to identify patients at risk for disease and the prevention of subsequent clinical events will need to be shown to be cost-effective to the system in both dollars and morbidity and mortality. As we have discussed here, the reality is that multimarker panels are currently available. Over the next several years, the academics will put the science through the rigors of clinical trials and treating physicians will determine whether these tests have a meaningful impact on identifying patients at risk and those who need escalating preventive therapies. Not all patients with positive markers will have disease and we will find that some markers have little utility, but we believe that through the early identification of risk and the aggressive implementation of preventive medicine, the implementation of multimarker panels hold the potential to prevent heart attacks and strokes in our patients.

Financial & competing interests disclosure

MS Penn is the scientific and medical founder of Cleveland Heart Lab, Inc. (OH, USA). As such, he is the inventor named on patent applications associated with a portion of the testing discussed and he receives royalties for these inventions. MS Penn also serves as the Chief Medical Officer of Cleveland Heart Lab, Inc. and, as such, receives consulting fees and equity. ABW Klemes is the Chief Medical Officer of MDVIP, Inc. for which she receives salary and equity. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1. Siegel RL, Ward EM, Jemal A. Trends in colorectal cancer incidence rates in the United States by tumor location and stage, 1992–2008. *Cancer Epidemiol. Biomarkers. Prev.* 21(3), 411–416 (2012).
2. McManus DD, Gore J, Yarzebski J, Spencer F, Lessard D, Goldberg RJ. Recent trends in the incidence, treatment, and outcomes of patients with STEMI and NSTEMI. *Am. J. Med.* 124(1), 40–47 (2011).
3. Agarwal U, Zhou X, Weber K, Dadabayev AR, Penn MS. Critical role for white blood cell NAD(P)H oxidase-mediated plasminogen activator inhibitor-1 oxidation and ventricular rupture following acute myocardial infarction. *J. Mol. Cell. Cardiol.* 50, 426–432 (2011).

▪ **Experimental studies demonstrated a hierarchy of leukocyte-generated oxidants.**

4. Kerekes G, Soltesz P, Nurmohamed MT *et al.* Validated methods for assessment of subclinical atherosclerosis in rheumatology. *Nat. Rev. Rheumatol.* 8(4), 224–234 (2012).
5. Brennan ML, Penn MS, Van Lente F *et al.* Prognostic value of myeloperoxidase in patients with chest pain. *N. Engl. J. Med.* 349(17), 1595–1604 (2003).

▪▪ **First demonstration of the clinical utility of myeloperoxidase for the identification of vulnerable plaque. Also showed that high-sensitivity CRP (hsCRP) does not identify vulnerable plaque.**

6. Salonen JT, Salonen R, Seppanen K, Rauramaa R, Tuomilehto J. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. *Circulation* 84(1), 129–139 (1991).
7. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N. Engl. J. Med.* 347(20), 1557–1565 (2002).
8. Ridker PM, Cannon CP, Morrow D *et al.* C-reactive protein levels and outcomes after statin therapy. *N. Engl. J. Med.* 352(1), 20–28 (2005).

▪▪ **Showed that escalating treatment in patients with high inflammatory marker, hsCRP, led to survival benefit, regardless of cholesterol levels.**

9. Ridker PM, Danielson E, Fonseca FA *et al.* Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N. Engl. J. Med.* 359(21), 2195–2207 (2008).

▪▪ **Demonstrated that LDL and hsCRP are additive risk factors in an apparently healthy population of women.**

10. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 105(9), 1135–1143 (2002).
11. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N. Engl. J. Med.* 336(14), 973–979 (1997).

▪ **One of the first studies to demonstrate the utility of hsCRP for identifying patients for enhanced treatment to prevent ischemic heart disease.**

12. Pedersen TR, Kjekshus J, Berg K *et al.* Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 344(8934), 1383–1389 (1994).
13. Roest M, Voorbij HA, van der Schouw YT, Peeters PH, Teerlink T, Scheffer PG. High levels of urinary F2-isoprostanes predict cardiovascular mortality in postmenopausal women. *J. Clin. Lipidol.* 2(4), 298–303 (2008).

14. Siamakpour-Reihani S, Scarbrough PM, Wang F *et al.* Systemic markers of oxidative status and colorectal adenomatous polyps. *Ann. Epidemiol.* 22(8), 587–591 (2012).

15. Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. *JAMA* 299(19), 2287–2293 (2008).

16. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators; *N. Engl. J. Med.* 342(3), 145–153 (2000); erratum in *N. Engl. J. Med.* 342(10), 748 (2000); *N. Engl. J. Med.* 342(18), 1376 (2000).

17. Kolodgie FD, Burke AP, Skorija KS *et al.* Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 26(11), 2523–2529 (2006).

18. Penn MS. The role of leukocyte-generated oxidants in left ventricular remodeling. *Am. J. Cardiol.* 101(10A), D30–D33 (2008).

19. Oemrawsingh RM, Lenderink T, Akkerhuis KM *et al.* Multimarker risk model containing troponin-T, interleukin 10, myeloperoxidase and placental growth factor predicts long-term cardiovascular risk after non-ST-segment elevation acute coronary syndrome. *Heart* 97(13), 1061–1066 (2011).

20. Heslop CL, Frohlich JJ, Hill JS. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. *J. Am. Coll. Cardiol.* 55(11), 1102–1109 (2010).

▪ **Study showed that myeloperoxidase predicted mortality in a patient population with mild coronary artery disease and further demonstrated that myeloperoxidase and hsCRP offered additive risk stratification.**

21. Wong ND, Gransar H, Narula J *et al.* Myeloperoxidase, subclinical atherosclerosis, and cardiovascular disease events. *JACC Cardiovasc. Imaging* 2(9), 1093–1099 (2009).

22. Brevetti G, Schiano V, Laurenzano E *et al.* Myeloperoxidase, but not C-reactive protein, predicts cardiovascular risk in peripheral arterial disease. *Eur. Heart J.* 29(2), 224–230 (2008).

23. Matusiak N, van Waarde A, Bischoff R *et al.* Probes for non-invasive matrix metalloproteinase-targeted imaging with PET and SPECT. *Curr. Pharm. Des.* (2013) (Epub ahead of print).