Correspondence

Thiobarbituric Acid Reactive Substances as a Biomarker for Coronary Heart Disease

To the Editor

In their recent article in *J AtherosclerThromb*, Tanaka *et al.*¹⁾ underlined the predictive value of markers of lipid peroxidation in coronary heart disease (CHD). In this large scale cohort study, 1945 apparently healthy individuals were followed up for a median of 10.9 years. During this period, 44 coronary events were documented. Serum level of thiobarbituric acid (TBA) reactive substances (TBARS), expressed in malondialdehyde (MDA) equivalent, was measured using the widely adopted method described by Yagi²⁾. After adjusting for other traditional risk factors, the level of TBARS was a significant and independent predictor of CHD. This study highlighted the critical role of lipid peroxidation in atherogenesis and subsequent progression to CHD.

The authors referenced the PREVENT (Prospective Randomized Evaluation of the Vascular Effects of Norvasc) trial, another large cohort study that examined the prognostic value of TBARS as a biomarker for cardiovascular events in stable coronary artery disease³⁾. In this study of 634 patients, those in the highest quartile of serum TBARS levels at baseline had a relative risk of 3.3 for major vascular events during the 3-year follow-up period. The investigators of the PREVENT study also assessed the predictive role of serum lipid hydroperoxides (LOOHs) in the same cohort. LOOHs are the primary products of lipid peroxidation and may undergo iron-mediated electron reduction and oxygenation to release other free radicals and trigger the chain events of lipid peroxidation. Similarly, patients in the highest quartile of baseline serum LOOH levels experienced a significantly higher risk of non-fatal vascular events during the follow-up period⁴⁾. Findings from the PREVENT trial and the current study by Tanaka *et al.* strongly support the predictive value of biomarkers of oxidative stress for adverse outcomes in atherosclerosis.

Amongst the biomarkers determining oxidative stress, the TBARS assay has been favoured by clinical studies because of the simplicity of its application⁵⁾. The principle of this assay involves heating biological samples with TBA under acidic conditions to allow the formation of MDA-(TBA)₂ adduct, a chromogen which may be measured either colourimetrically or fluorometrically⁶⁾. In their discussion, the authors aptly drew attention to an issue of specificity related

to the TBARS assay. In particular, different aldehydes can be formed in the lipid peroxidation process and aldehydes other than MDA may form chromogenic complexes with TBA, which exhibit similar absorbance or emission wavelengths. Several other biological compounds, including sugars, amino acids and bilirubin, are also reactive towards TBA and form TBA reactive materials which confound the measurement by the conventional TBARS assay⁷⁾. More importantly, MDA is also a by-product of cyclooxygenase activity in platelets⁸⁾; therefore, the measurement of MDA in serum may lead to an over-estimation due to the formation of MDA formation *ex-vivo*, as platelet activation is required for the formation of serum⁹⁾.

When using serum as the sample matrix for analysis of oxidative stress, it is worth considering the issue of specificity for this particular reason. For example, in the PREVENT trial, the authors attempted to address the issue of specificity of TBARS measurement in serum by subjecting the samples to a two-stage extraction process. Serum samples were first treated in the classic thermoreaction TBARS assay, followed by separation of TBARS using reverse phase high performance liquid chromatography (HPLC) to exclude other TBA reactive materials which may have the same absorbance/emission wavelengths as the MDA-TBA₂ adduct³⁾. This was achieved by coupling a spectrophotometric detector at 532 nm and a fluorescence detector (excitation=515 nm, emission=553 nm) on a 150 mm×4.6 mm C18 column with 5 µm particle size. The mobile phase consisted of 80% phosphate buffer (10 mM, pH 5.8) with 20% methanol and the flow rate set to 1 mL/min. However, the additional HPLC separation step is time consuming and it is logistically challenging to apply it to clinical studies involving a large number of patients.

One alternative to improve the specificity of the TBARS assay in clinical samples may be to use plasma instead of serum samples, as this can minimise the interference of other aldehydes produced *ex-vivo* by platelets via the cyclooxygenase pathway, as discussed earlier. Further, the use of a chelating agent (such as EDTA) as an anticoagulant in the preparation of plasma may further reduce the variability of the TBARS assay due to the iron content of reagents used in the analysis¹⁰⁾.

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References

- 1) Tanaka S, Miki T, Sha S, Hirata K, Ishikawa Y, Yokoyama M: Serum levels of thiobarbituric acid-reactive substances are associated with risk of coronary heart disease. *J Atheroscler Thromb*, 2011; 18: 584-591
- 2) Yagi K: A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med*, 1976; 15: 212-216
- 3) Walter MF, Jacob RF, Jeffers B, Ghadanfar MM, Preston GM, Buch J, Mason RP: Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: A longitudinal analysis of the prevent study. *J Am Coll Cardiol*, 2004; 44: 1996-2002
- 4) Walter MF, Jacob RF, Bjork RE, Jeffers B, Buch J, Mizuno Y, Mason RP: Circulating lipid hydroperoxides predict cardiovascular events in patients with stable coronary artery disease: The prevent study. *J Am Coll Cardiol*, 2008; 51: 1196-1202
- 5) Holley AE, Cheeseman KH: Measuring free radical reactions in vivo. *Br Med Bull*, 1993; 49: 494-505
- 6) Kosugi H, Kato T, Kikugawa K: Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Anal Biochem*, 1987; 165: 456-464
- 7) Meagher EA, FitzGerald GA: Indices of lipid peroxidation in vivo: Strengths and limitations. *Free Radic Biol Med*, 2000; 28: 1745-1750
- 8) Makris PE, Tsakiris DA, Papadopoulos A, Ballas A: The ratio mda/mdaa as a new index of platelet hyperactivity. *Haemostasis*, 1985; 15: 331-336
- 9) Buller HR, Ten Cate T: Coagulation and platelet activation pathways. A review of the key components and the way in which these can be manipulated. *Eur Heart J*, 1995; 16 Suppl L: 8-10
- 10) Duthie GG, Morrice PC, Ventresca PG, McLay JS: Effects of storage, iron and time of day on indices of lipid peroxidation in plasma from healthy volunteers. *Clin Chim Acta*, 1992; 206: 207-213

Regent Lee and Charalambos Antoniades

Department of Cardiovascular Medicine, University of Oxford, UK

Address for correspondence: Regent Lee, Department of Cardiovascular Medicine, Level 6, West Wing, John Radcliffe Hospital, Headington, Oxford, UK
E-mail: regent.lee@cardiov.ox.ac.uk