MECHANICAL AND CIRCULATING BIOMARKERS IN ISOLATED CLINIC HYPERTENSION

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SUMMARY

1. This review examines the current evidence for altered mechanical and circulating biomarkers in isolated clinic hypertension and their potential significance.

2. Arterial stiffness, as assessed by central pulse wave velocity, is influenced by multiple cardiovascular risk factors; however, an independent association with isolated clinic hypertension (ICHT) has not been convincingly shown in four small studies.

3. Endothelial dysfunction, as assessed by brachial artery flow-mediated vasodilation, circulating levels of endothelial markers (e.g. nitrite/nitrate, von Willebrand factor, endothelin-1) and/or circulating levels of inhibitors of vascular nitric oxide (plasma asymmetric dimethylarginine, homocysteine), has been shown to be present in established hypertension and to a variable and inconsistent extent in subjects with ICHT.

4. Evidence of increased oxidative stress in ICHT versus normotensive subjects was found in two of three studies.

5. Circulating inflammatory markers C-reactive protein and plasminogen activator inhibitor-1 were significantly increased in two of three and two of two studies, respectively, in ICHT compared with normotensive subjects.

6. Urinary albumin excretion is a marker of both arterial and renal disease. The consensus from seven studies in patients with ICHT is that albuminuria is not an independent marker for ICHT.

7. Studies to date assessing biomarkers in ICHT have been small and cross-sectional. Larger, long-term longitudinal studies of arterial functional and circulating biomarkers are required to assess the potential vascular impact of ICHT.

Key words: arterial stiffness, endothelial dysfunction, inflammation, isolated clinic hypertension, oxidative stress.

INTRODUCTION

Blood pressure measured in the doctor’s office or clinic has been the most established biomarker of future cardiovascular disease, but it is no longer sufficient if used as the sole method of assessing the usual blood pressure of an individual. Twenty-four hour ambulatory blood pressure and home blood pressure monitoring have identified the discrepancy that exists between clinic and out-of-clinic blood pressures in a significant number of patients and in these patients, with either isolated clinic or masked hypertension, risk calculations based on clinic readings may lead to erroneous risk prediction of cardiovascular risk and inappropriate treatment.

Isolated clinic hypertension (ICHT), also known as ‘white-coat’ hypertension, is a common clinical condition, where clinic measurements are elevated (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg) but home and 24 h ambulatory blood pressure measurements are within the normotensive range (day average SBP < 135 mmHg and day average DBP < 85 mmHg). The prevalence depends on blood pressure cut-off levels and accurate classification requires more than one 24 h blood pressure recording.1 There has been considerable debate about the significance of the condition in predicting future cardiovascular disease.2 In an 8-year follow-up period in the Ohasama study, ICHT was identified as a significant predictor for the development of hypertension as assessed by home measurement1 confirming the results of an earlier study.4 Recently, Verdeccchia et al.6 described a trend towards increasing incidence of stroke, with the hazard curve for isolated clinic hypertension crossing that of the established hypertensive group at 9 years of follow-up.

Over the past 10 years there has been a concerted effort to explore the clinical usefulness of surrogate markers of arterial function. There have been many indices proposed for this purpose, including those indicative of arterial stiffness in central or peripheral arterial segments (pulse wave velocity, stiffness index), indices of arterial compliance (systemic arterial compliance, distensibility) and a composite measure of wave reflection and systemic arterial stiffness (aortic augmentation index). Dynamic or functional influences such as endothelial-mediated changes can influence these indices, but the scope of these dynamic components has generally not been well characterized. Central (aorto-femoral) pulse wave velocity (PWV) is the index of arterial stiffness with most promise as a functional biomarker.8 Other surrogate non-mechanical biomarkers of cardiovascular disease can be broadly classified into three categories: measures of endothelial function, circulating inflammatory markers and markers of oxidative stress (Fig. 1). In established hypertension there is good evidence for disturbed vascular
function and structure as shown by changes in each of these biomarkers.

This review examines the evidence for alteration in biomarkers of vascular disease in ICHT.

**ARTERIAL STIFFNESS**

Central PWV in a variety of patient groups and in normal individuals has consistently been shown to be an independent predictor of cardiovascular outcome; it tends to track appropriately with disease severity and there is a strong case for its inclusion in risk assessment algorithms. An age-adjusted reference curve for PWV has recently been reported with demonstrable construct validity in regards to identification of groups at medium and high risk of cardiovascular disease.

Four studies of PWV measurement in patients with ICHT are reported (Fig. 2). Only two of the studies used central (carotid/aortic to femoral) PWV, which is now generally accepted as the best mechanical predictor of cardiovascular disease. Tillin et al. reported that central (carotid-femoral) PWV was significantly associated with coronary artery calcification score and with carotid or femoral artery intima-media thickness, whereas carotid-radial PWV and femoral-posterior tibial PWV were not. Pannier et al. showed that only central PWV, not brachial artery or femoro-tibial PWV, was able to predict cardiovascular mortality in patients with end-stage renal failure.

Silva et al. showed that, unadjusted for concomitant risk factors, there was a progressive increase in mean carotid-femoral PWV from a normotensive to an ICHT group and then to an established hypertensive group. Ribeiro et al. found that patients with ICHT had significantly higher aorto-femoral PWV compared with normotensive subjects, but that this was influenced by the number of coexisting risk factors. In patients at low overall risk, with one or no cardiovascular risk factors, this difference was not significant. A recently reported study in Japanese subjects used radial-femoral PWV measurements and showed that after adjustment for age, gender, body mass index (BMI), habitual alcohol drinking, lifetime smoking and SBP during PWV measurement, there was no significant difference between normotensive and ICHT subjects. Longo et al. used carotid to radial PWV, found a significant difference between the ICHT group and the normotensive group after adjusting for age, sex, heart rate, weight, height, blood pressure, smoking, alcohol use, physical activity, fasting glucose, total cholesterol and triglycerides. However this should be interpreted with caution given the lack of outcome predictability of this measure as discussed above. There have been no long-term studies of ICHT that have looked at baseline central PWV as an outcome predictor.

**BIOMARKERS OF ENDOTHELIAL FUNCTION**

Vascular endothelial cells have multiple, often counter-regulatory activities relating to vasodilatation and vasoconstriction, thrombosis and fibrinolysis, platelet aggregation and adhesion, leucocyte adhesion and activation, smooth muscle cell proliferation and migration, immunological and inflammatory processes. Nitric oxide (NO) is produced by endothelial cells in response to various stimuli, with shear-stress being the most important. It has been estimated that NO tonically restrains BP in humans by approximately 30 mmHg.
Shear-stress induced NO can be assessed by the technique of brachial arterial flow-mediated dilation (FMD) in response to postischaemic reactive hyperaemia, to determine a measure of endothelium-dependent dilation (EDD). This is impaired in established hypertension. Gomez-Cereza et al. reported a linear relationship between 24-h SBP and EDD ($r = -0.48$, $P = 0.0001$), with significant differences between ICHT and normotensive groups.

Although not statistically significant, there were higher proportions of females (57% versus 33%) and overweight subjects (57% versus 36%) in the ICHT group. Pierdemenico et al. found no significant difference in EDD between ICHT and normotensive subjects (Fig. 3).

Plasma levels of NO can be estimated by levels of its metabolites, NO$_2$ and NO$_3$. Karter et al. showed that mean plasma nitrite/nitrate was reduced in ICHT compared with normotensive controls (42 ± 2 versus 48 ± 6 µmol/L, Fig. 3) and further reduced in established hypertension (32 ± 3 µmol/L, $P < 0.001$). Curgunlu et al. reported very similar results, with the same numbers of subjects and likely from the same population. Pierdemenico et al. found no significant difference in EDD between ICHT and normotensive subjects (Fig. 3).

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Von Willebrand factor (vWF) is a glycoprotein, required for platelet aggregation and adhesion and for factor VIII survival. It is stored in endothelial cells and secreted into the plasma. Increased vWF levels are associated with hypertension and progression of cardiovascular disease. Two studies have examined vWF levels in groups of normotensive, ICHT and established hypertensive subjects. Both showed increased vWF levels in groups of subjects with established hypertension, but only the study by Coban et al. showed a difference between ICHT and normotensive groups (Fig. 3). In that study groups were matched for age, gender and body mass index. Kario et al. studied an older population, in which groups were age-matched, but not matched for other factors.

Endothelin-1 (ET-1) is a potent vasoconstrictor expressed by endothelial cells in response to a number of stimuli and has been found to be elevated in hyperlipoproteinaemia, insulin resistance, diabetes, smokers and obese subjects with metabolic syndrome. High concentrations have also been found in atherosclerotic plaques. Plasma levels of ET-1 are normal in most subjects with essential hypertension but are elevated in other types of hypertension such as renal hypertension. Plasma ET-1 and vascular endothelial growth factor levels were increased in subjects with ICHT compared with normotensive controls in the study of Karter et al. Urinary ET-1 excretion was reported to be increased in male adolescent ICHT subjects compared with male adolescent normotensives. Although the male ICHT group had a higher mean BMI, the difference persisted after adjustment for BMI. No difference was found in females. Potential confounders other than BMI were not reported.

**INHIBITORS OF VASCULAR NO**

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthesis. ADMA levels are elevated in chronic renal disease, hypercholesterolaemia, hyperhomocysteinaemia and hypertension. ADMA synthesis is triggered by shear stress and this is the likely
cause for increased levels in hypertension. It is inversely related to endothelial function in hypertension as measured by peak haemo-
dynamic response to acetylcholine. 27 Oxidative stress may also increase ADMA levels by inhibiting dimethylaminohydrolase
(DDAH), which metabolises ADMA to citrulline. 28 As shown in
Fig. 4 only one study was found that measured ADMA levels in 
ICH subjects. 21 Mean ADMA level in ICHT subjects was statistically
increased when compared with the normotensive group, but less than
that of the established hypertensive group (3.21 ± 0.49 versus
2.84 ± 0.58 versus 4.24 ± 0.38).

Increased plasma total homocysteine is associated with increased risk of cardiovascular disease, particularly in hypertensive subjects. Three studies have compared mean plasma homocysteine levels in ICHT and normotensive subjects; 21,29,30 in two of these levels were significantly higher in ICHT subjects (Fig. 4). However these studies did not adjust for potential confounding factors, such as renal function.

**BIOMARKERS OF OXIDATIVE STRESS**

Oxidative stress occurs when reactive oxygen species (ROS) react with and damage tissue and organs. It is hypothesized that oxidative stress plays a role in the pathogenesis of hypertension. 31 but this has been challenged. 32 Results of three studies that have examined various markers of oxidative stress in ICHT are summarized in
Table 1. 33–35 Two of the three studies reported evidence of increased oxidative stress in ICHT compared with normotensive controls. 34,35

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**Table 1** Oxidative markers and anti-oxidant activity

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EHT, essential hypertension; FPL, fluorescent products of lipid peroxidation in native LDL; GSH, glutathione; ICHT, isolated clinic hypertension; lag phase, a measure of LDL resistance to oxidation in vitro; LDL, low-density lipoprotein; MDA, malondialdehyde; NS, not significant at the 5% level; NT, normotension; oxLDL, oxidized low-density lipoprotein; PCO, protein carbonyls; PON1, paraoxonase; P-SH, protein thiol; PR, peroxidation rate which is an indication of the autocatalytic chain reaction of lipid peroxidation after depletion of anti-oxidant content; SOD, superoxide dismutase.

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**CIRCULATING INFLAMMATORY MARKERS**

Inflammation is well established as a major contributing factor in atherogenesis. Cardiovascular risk factors, including smoking, obesity, hypertension, hyperglycaemia and atherogenic lipoproteins all cause injury to the vascular endothelium, which initiates a local inflammatory response. The inflammatory biomarker that is most extensively studied is C-reactive protein (CRP), an acute phase protein considered the most sensitive circulating marker of inflammation. CRP has been associated with cardiovascular events 36,37 and has been suggested as an independent risk marker for cardiovascular disease. 38 Recent evidence suggests that CRP itself is deleterious to the vascular endothelium: CRP induces coronary and aortic endothelial release of inflammatory cytokines 39 increases expression of angiotensin II type 1 receptors in vascular smooth muscle cells, 40 reduces production of NO and increases the uptake of low-density

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lipoprotein (LDL) by macrophages. CRP has been found in other prospective studies to be an independent predictor for the development of hypertension suggesting that inflammation may precede hypertension and endothelial dysfunction, but causal evidence is yet to be established.

Results of studies comparing inflammatory markers in ICHT and normotensive subjects are shown in Fig. 5. Three studies were identified that compared CRP in ICH subjects in comparison to normotensives and sustained hypertensives. These studies used similar definitions of ICH and were matched for age and gender. Two of the studies showed significant difference in CRP (between ICH and normotensive groups).

Plasminogen activator inhibitor-1 (PAI-1) is a marker of impaired fibrinolysis and atherothrombosis. PAI-1 is induced by CRP. Both PAI-1 and CRP are increased in obesity, diabetes and the metabolic syndrome. PAI-1 is also found in platelets and can be released during blood taking. PAI-1 levels are elevated in established hypertension and PAI-1 levels were found to be associated with
blood pressure levels in children with hypertensive parents. In a study of the Framingham population both CRP and PAI-1 were found to be independent markers of incident hypertension.

Two studies were identified that compared PAI-1 in ICHT subjects compared to normotensives and sustained hypertensives. Both studies report a significant difference between PAI-1 levels in the ICHT group and the normotensives group.

**URINARY ALBUMIN EXCRETION**

Seven studies have reported urinary albumin excretion in ICHT. Although increased urinary albumin excretion was reported in ICHT, there was no significant difference between ICHT and normotensives groups.

**CONCLUSIONS**

Established hypertension is associated with arterial stiffness, endothelial dysfunction, increased vascular oxidative stress and an increase in circulating inflammatory markers. It is likely that ICHT is a prehypertensive state that requires close monitoring. This review of many cross-sectional studies provides suggestive evidence for altered vascular function in ICHT, as shown by arterial endothelial dysfunction and circulating markers of oxidative stress and inflammation, but arterial stiffness is not increased. There is a need for large, long-term longitudinal studies of arterial functional and circulating biomarkers to assess the potential vascular effect of ICHT. These studies need to take into account the multiple variables that can influence these biomarkers.

**REFERENCES**