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Review

The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation
Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society

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ABSTRACT

While risk scores are invaluable tools for adapted preventive strategies, a significant gap exists between predicted and actual event rates. Additional tools to further stratify the risk of patients at an individual level are biomarkers. A surrogate endpoint is a biomarker that is intended as a substitute for a clinical endpoint. In order to be considered as a surrogate endpoint of cardiovascular events, a biomarker should satisfy several criteria, such as proof of concept, prospective validation, incremental value, clinical utility, clinical outcomes, cost-effectiveness, ease of use, methodological consensus, and reference values. We scrutinized the role of peripheral (i.e. not related to coronary circulation) noninvasive vascular biomarkers for primary and secondary cardiovascular disease prevention. Most of the biomarkers examined fit within the concept of early vascular aging. Biomarkers that fulfill most of the criteria and, therefore, are close to being considered a clinical surrogate endpoint are carotid ultrasonography, ankle-brachial index and carotid-femoral pulse wave velocity; biomarkers that fulfill some, but not all of the criteria are brachial ankle pulse wave velocity, central haemodynamics/wave reflections and C-reactive protein; biomarkers that do no not at present fulfill essential criteria are flow-mediated dilation, endothelial peripheral arterial tonometry, oxidized LDL and dysfunctional HDL. Nevertheless, it is still unclear whether a specific vascular biomarker is overly superior. A prospective study in which all vascular biomarkers are measured is still lacking. In selected cases, the combined assessment of more than one biomarker may be required.

1. Introduction

Accurate assessment of cardiovascular (CV) risk is essential for clinical decision-making; the benefits, risks and costs of management strategies must be weighed to choose the best individually tailored preventive strategy. Many scores have been developed over the years to classify patients into low, medium or high CV risk groups. In Europe, the HeartScore and its online version, Systemic Coronary Risk Evaluation (SCORE), have been introduced for the prediction of the total fatal 10-year CV risk [1]. In the U.S.A., a number of different risk scores have been validated. The most widely used ones come from the Framingham Heart Study group that has proposed an array of 14 risk equations [2], while the most recently proposed risk equation by the American College of Cardiology/American Heart Association (ACC/AHA) [3] has been the subject of controversy.

Risk scores are not, however, flawless and their head-to-head comparison opens many questions [4]. Moreover, a small, yet significant gap exists between predicted and actual event rates, leading to under- and over-prediction, thus raising the issue of calibration. The extrapolation to populations different from the original cohort, the choice of traditional risk factors that are included, the changes in population characteristics because of the time delay between observational studies and application of risk scores, as well as the omission of novel indices relating to CV pathophysiology may partly explain the limitations of risk scores [5].

Additional tools to further stratify the risk of patients are biomarkers. According to the National Institutes of Health definition, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [6]. In essence, and in the setting of prevention, CV biomarkers reflect early functional or morphological changes, well before overt disease manifests. This identification of subclinical disease may open a window of opportunity to prevent the occurrence of clinical CV disease by timely treatment.

Vascular biomarkers as surrogate endpoints:

A surrogate endpoint is a biomarker that is intended as a substitute for a clinical endpoint. Changes in surrogate endpoints are detected earlier and at a lower cost than clinical endpoints (e.g. morbidity and mortality). Thus, diagnosis and clinical trials are facilitated. A surrogate endpoint is expected to predict clinical benefit/harm or lack thereof, based on epidemiologic, therapeutic, pathophysiological or other scientific evidence. In order to be considered as a "surrogate endpoint" of CV events, a biomarker should satisfy several steps. According to the AHA, the 6 phases of evaluation of a novel risk marker include: **1. *Proof of concept:*** Do novel biomarker levels differ between subjects with and without outcome? **2. *Prospective validation:*** Does the novel biomarker predict development of future outcomes in a prospective cohort or nested case-cohort study? **3. *Incremental value:*** Does it add predictive information over and above established, standard risk markers? **4. *Clinical utility:*** Does it change predicted risk sufficiently to change recommended therapy? **5. *Clinical outcomes:*** Does the use of the novel biomarker improve clinical outcomes, especially when tested in a randomized clinical trial? **6. *Cost-effectiveness:*** Does the use of the biomarker improve clinical outcomes sufficiently to justify the additional costs? [7].

On top of the aforementioned criteria, a biomarker should be relatively easy to measure, in a non-invasive manner, according to a well-defined protocol and the obtained metric should distinguish individuals at risk, in order to be deemed suitable for use in clinical practice. Therefore, 3 additional steps (not present in the AHA scientific statement) [7] should be added in the assessment of vascular biomarkers to qualify as *clinical* surrogate endpoints: **7. *Ease of use:*** this will allow widespread application, **8. *Methodological consensus:*** necessary to allow comparisons between studies and **9. *Reference values:*** or, at least, cut-off values.

Fulfilment of the 9-step criteria for each vascular biomarker (Table 1) will be subsequently discussed in relevant sections. Though all have a sound proof of concept and have been prospectively validated (steps 1 and 2), the incremental value, clinical utility and clinical outcomes (steps 3–5) have only been established for few of them; cost-effectiveness data (step 6) are almost uniformly lacking. The majority of them are easily measured (step 7), methodological consensus (step 8) has been reached for some, yet reference values (step 9) exist for only a few vascular biomarkers.

Most of the biomarkers analyzed in this document fit within the concept of **early vascular aging**. According to this concept, a cumulative measure of the impact of CV risk factors on the arterial wall, in association and interaction the individual genetic background, has the potential to accurately gauge a person's overall CV risk [8]. The aim of this document is to scrutinize the role of peripheral (i.e. not related to coronary circulation) noninvasive vascular biomarkers for primary and secondary CV disease prevention. The most widely used biomarkers related to vascular structure and function are presented; a uniform approach has been followed, including a brief methodological presentation, the current role, potential use as a surrogate endpoint, advantages/disadvantages and future perspectives. Regarding the order of presentation, the date of publication of the first outcome study for each vascular biomarker was used; those with the oldest outcome studies are presented first. The role of vascular biomarkers in the setting of comorbidities is presented, as well as their potential for guiding treatment. The Tables, as well as a dedicated section of the document provide detailed comparisons between vascular biomarkers at a glance; these should help clinicians in selecting the appropriate index to be measured in different clinical and research scenarios.

2. Carotid ultrasonography

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **IIa/A**.

Infiltration of the subintimal layer of arteries by lipids and inflammatory cells constitutes an early process in the atherosclerotic continuum. Imaging techniques with adequate spatial resolution can assist in the timely detection and quantification of structural changes in the arterial wall that are associated with atheroma, fibrosis and the aging process. A thick intima-media complex, and carotid plaque as its extreme manifestation, serves as a proxy of generalized atherosclerosis. Though often regarded as two distinct phenotypes and biomarkers with different usefulness for risk prediction, both carotid intima-media thickness (cIMT) and plaque presence are assessed during imaging and provide complementary prognostic information, thus they cannot be viewed in isolation. The most commonly and easily accessed site is the common carotid artery and its bifurcation, though the femoral artery has also been studied.

Methodology:

cIMT is measured with transcutaneous ultrasound in B-mode images of the carotid tree as the distance of the intimal to the adventitial layer (visible as a typical double line of the arterial wall). cIMT is measured most easily in the segment of the distal common carotid artery based on the Mannheim consensus [9], but risk prediction should be based on a thorough scan of the carotid arteries to detect the presence of plaques and on the measurement of the cIMT of the common carotid artery [10]. The intra- and inter-observer agreement, as well as the accuracy of measurements of the mean cIMT was found to be highest in the common carotid artery, followed by the carotid bifurcation and inner carotid artery [11–15]. The optimal reproducibility obtained by scanning the common carotid artery is due to the ease of acquiring good quality images at an insonification angle of 90°; this, however, comes at the cost of missing disease that more often develops at the carotid bulb/internal carotid artery [16]. Reading should be done with semi-automated reading software (better than automated) [17] over a 1-cm segment because of the ease of use, potential to improve ultrasound quality, and probably lower variability compared to manual measurements [15,18,19]. An alternative method with better spatial and temporal resolution is the measurement of cIMT using echo-tracking [20], however, a possible advantage of B-mode based analysis is that it can be easily used with standard high-resolution ultrasound equipment. The reproducibility of echo-tracking and B-mode based measurement of cIMT was shown to be similar in patients with increased risk factor burden and/or manifest atherosclerotic disease [21,22]. A value above the 75th percentile of a reference population is generally accepted as a threshold for increased cIMT [10]. A value of 0.9 mm is set as a cut-off in the European Society of Cardiology (ESC) guidelines [23,24]; this simplification may, however, lead to misclassification in different populations and age groups where similar values may be within the normal range [25].

Carotid plaque may represent a later stage or a different phenotype of atherosclerosis than increased cIMT [9,26]. However, this cannot be differentiated by ultrasound; many studies do not discriminate between plaque or cIMT and incorporate plaque thickness into cIMT measurements [27]. As a consequence, maximum cIMT (in contrast to mean cIMT) is close to plaque thickness if the Mannheim consensus is not used and cIMT is considered as a continuous parameter; this causes some confusion in the interpretation of the studies. Carotid plaque may be measured with ultrasound in 2D and in 3D-images. Several characteristics of plaques are currently examined, like the dissemination within the carotid tree, echogenicity (echolucent, echogenic, mixed echogenicity), echogenic distribution pattern (homogeneous versus inhomogeneous) and evaluation of surface structure (regular versus irregular [28]). Recently, plaque vascularization on contrast-enhanced ultrasound has been developed to optimize cardiovascular risk prediction [29]. Among these plaque characteristics, total plaque volume was the most commonly evaluated parameter showing good inter- and intra-observer reproducibility (>90%) [28,30]. However, it is unclear whether different 3D techniques could be sufficiently standardized between laboratories, thus allowing widespread clinical usage [27]. Robust data upon superiority of 3D ultrasound over 2D is pending [28]. At present, the assessment of carotid plaque in population studies is mainly done in B-mode images of the carotid tree. In the clinical setting, easy to assess and reproducible plaque-based parameters (presence vs. absence or number of plaques), and simple definitions (plaque: maximum cIMT >1.5 mm) are advocated [31]. At the moment, no

Table 1
Criteria for vascular biomarkers to qualify as clinical surrogate endpoints.

1	***Proof of concept***	Do novel biomarker levels differ between subjects with and without outcome?
2	***Prospective validation***	Does the novel biomarker predict development of future outcomes in a prospective cohort or nested case-cohort study?
3	***Incremental value***	Does it add predictive information over and above established, standard risk markers?
4	***Clinical utility***	Does it change predicted risk sufficiently to change recommended therapy?
5	***Clinical outcomes***	Does the use of the novel biomarker improve clinical outcomes, especially when tested in a randomized clinical trial?
6	***Cost-effectiveness***	Does the use of the biomarker improve clinical outcomes sufficiently to justify the additional costs?
7	***Ease of use***	Is it easy to use, allowing widespread application?
8	***Methodological consensus***	Is the biomarker measured uniformly in different laboratories? Are study results directly comparable?
9	***Reference values (or cut-off values)***	Are there published reference values, or, at least, cut-off values?

Modified from: Hlatky et al. Circulation 2009. Criteria 7–9 constitute additional essential criteria to the original criteria 1–6 proposed by Hlatky and coworkers [7].

generally accepted continuous parameter has been introduced into guidelines.

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:*

cIMT: Multiple studies – by far more than for any other imaging biomarker - have shown higher cIMT in subjects with higher risk factor burden or manifest atherosclerotic disease [9].

Carotid plaque: A similar relation is true for carotid plaque but in a far lower number of publications [27,31] (Table 2).

2. *Prospective validation and 3. Incremental value:*

cIMT: The prediction of future outcomes in prospective cohorts has been proved in several studies (Table 3). A meta-analysis of 16 studies with 36,984 participants showed an increase in risk for future CV events of 16% per 0.1 mm difference in baseline cIMT [32].

A recent meta-analysis based on data from 14 cohort studies (45,828 individuals from the general population) showed that common cIMT measurements do not add significantly to the Framingham Risk Score (FRS) regarding the prediction of first-time myocardial infarction or stroke [33]. Another study did not show any predictive value of common cIMT measurement in subjects with diabetes mellitus [34]. However, it should be mentioned that this holds true only for common cIMT. Data for carotid bifurcation and internal cIMT have not been analysed so far. Furthermore, different definitions of the common carotid segment and tracing methods might have weakened the power of the analysis; in addition, the study population consisted mainly of Caucasian individuals, so results cannot be extrapolated to other ethnicities.

Carotid plaque: Carotid plaque confers a superior diagnostic accuracy for future myocardial infarction compared to cIMT according to a recent meta-analysis (n = 54,336) [27] and this was corroborated by a systematic review [35]. The presence of carotid plaques predicted CV mortality independently of the SCORE stratification and increased the risk for CV death by 2–4 times in intermediate risk and low risk individuals, respectively [36]. An incremental CV disease risk prediction with increasing number of plaques (1 site = 1.5; 95% confidence interval [CI]: 1.0–2.2; ≥2 sites = 2.2; 95% CI: 1.6–3.1), but not with increasing cIMT has been shown in the Three-City Study [37]. In the Atherosclerosis Risk In Communities (ARIC) study, both cIMT and presence of plaque contributed to improved CV disease risk prediction [38]. Similarly, ultrasound-derived plaque metrics improved risk prediction in the Multiethnic Study of Atherosclerosis (MESA) cohort [31]. In general, studies on carotid plaque are in smaller cohorts than those on cIMT; similarly, they consist of Caucasians mostly, so again results cannot be extrapolated to other ethnicities.

4. *Clinical utility:*

cIMT: The meta-analysis by Den Ruijter et al. demonstrated that cIMT had a small, yet significant potential for reclassification only in intermediate risk individuals, with a clinical net reclassification index (NRI) of 3.2% in men and 3.9% in women (overall NRI: 0.8%), with a wide range among studies [33]. Given the small reclassification potential of cIMT, which is only evident in intermediate risk individuals, its clinical utility is open to question.

Carotid plaque: In contrast to cIMT, studies that include assessment of plaque in general improve reclassification; the NRI for plaque presence was slightly higher and ranged from 5.6% for individuals with prior CV disease to 8.1% for individuals without CV disease at baseline [37]. In the MESA cohort, cIMT plus plaque together resulted in an overall NRI of 9.9%; when only intermediate risk subjects were considered, the clinical NRI was 21.7% [31]. Similar results were reported from the Framingham Offspring Study cohort and a large Chinese population [39,40] (Table 3).

5. Clinical outcomes: The question if a cIMT-driven therapy leads to better outcomes has not been answered yet. In practice, high cIMT values and/or presence of plaque are considered as target organ damage and call for aggressive risk factor modification. Though there are no studies to support such practices, carotid ultrasound scanning for detection of increased cIMT or carotid plaque may lead to increased prescription of aspirin and statins by physicians [41] and intensified goal setting for LDL-cholesterol levels [42]. However, conflicting results have been reported regarding the impact of carotid ultrasound scanning on patient motivation [41,43]. Lipid-lowering drugs can reduce carotid plaque volume [44]; nevertheless, cIMT and plaque are biomarkers that change slowly over time, therefore patients may be on optimal medical therapy for a prolonged period of time and, yet, have increased cIMT values and/or plaques. In spite of the aforementioned issues, cIMT has been used as an intermediate endpoint in randomised clinical trials of novel drugs [45]. Importantly, it has been demonstrated that cIMT changes do not have prognostic implications; in a large meta-analysis of 36,984 participants, cIMT progression was not linked to future CV events [32].

6. *Cost-effectiveness:*

cIMT: One study simulated the cost-effectiveness of cIMT screening over a 20–30 year period on the basis of the ARIC study data using a Markov model [46]. Based on a 1% lower absolute risk of myocardial infarction in men and of 1–3% lower risk in women respectively, cIMT measurements increased quality-adjusted life years (QALYs) by 0.01–0.02 in men and 0.03–0.05 in women. The corresponding costs were an additional $100 per man, and a cost-saving of $200–300 per woman.

Carotid plaque: No studies on cost-effectiveness for

Table 2
Sensitivity to detect changes and guide for pharmacological treatment.

	Guide for pharmacological therapy	Sensitivity for changes	Time to change	Prognostic value of changes
Carotid ultrasonography	++	Low	Slow	No
Ankle-brachial index	++	Low	No data	Moderate
Arterial stiffness				
Carotid-femoral pulse wave velocity	+++	High	Moderate	Moderate
Brachial-ankle pulse wave velocity	++	High	Moderate	No data
Central haemodynamics/Wave reflections	+++	High	Fast	Good guide for therapy, with the exception of patients with heart failure and a low ejection fraction
Endothelial function				
Flow mediated dilatation	+++	Very high	Fast	Moderate
Endothelial peripheral arterial tonometry	+	Very high	Fast	No data
Circulating biomarkers related to vascular wall biology				
High sensitivity C- reactive protein	+++	Moderate	Fast	No data

Table 3
Risk prediction improvement.

	Study	Outcome	Markers added	Δ C-statistic[a]	Overall net reclassification index	Clinical net reclassification index[b]	Reference
Carotid ultrasonography	USE-IMT meta-analysis (n = 45,828)	First time MI or stroke	Common cIMT	0.757 → 0.759	0.8%	3.6%	Den Ruijter et al., 2012
	Framingham Offspring Study (n = 2965)	Incident CVD	Mean common cIMT, maximum internal cIMT, plaque presence	Non-significant for mean common cIMT, 0.009 for maximum internal cIMT, 0.014 for plaque presence	Non-significant for mean common cIMT, 7.6% for maximum internal cIMT, 7.3% for plaque presence	12.7% for maximum internal cIMT	Polak et al., 2011
	Prog-IMT meta-analysis (n = 36,984)	Future CV events	Mean common cIMT	–	–	–	Lorenz et al., 2012
	Shijingshan district and Peking University community cohort (n = 3258)	Future CV events	Carotid plaque	–	–	–	Xie et al., 2011
				0.742 → 0.751	10.5%		
Ankle-brachial index	Multiethnic Study of Atherosclerosis (n = 1330)[c]	Incident CHD, CVD	ABI	0.623 → 0.650 (CHD), 0.623 → 0.650 (CVD)	3.6% (CHD), 6.8% (CVD)	3.6% (CHD), 6.8% (CVD)	Yeboah et al., 2012
	Ankle Brachial Index Collaboration meta-analysis (n = 48,294)	Total mortality	ABI	0.646 → 0.655 (men), 0.605 → 0.658 (women)	–		Ankle Brachial Index Collaboration 2008
Arterial stiffness							
Carotid-femoral pulse wave velocity	Individual data meta-analysis (n = 17,635)	Total/CV mortality, CV/CHD events, stroke	Log (aortic PWV)	–	8.34% (CV mortality)	24.27% (CV mortality)	Ben-Shlomo et al., 2013
	Meta-analysis (n = 15,877)	Total/CV mortality, CV events	Carotid-femoral PWV	–	–	–	Vlachopoulos et al., 2010
Brachial-ankle pulse wave velocity	Meta-analysis (n = 8169)	CV events	Brachial-ankle PWV	–	–	–	Vlachopoulos et al., 2012
Central haemodynamics/ Wave reflections	Multiethnic Study of Atherosclerosis (n = 5960)	CV events	PPA, RM, AIx	0.002 (PPA), 0.002 (RM)	10% (PPA), 15% (RM)	–	Chirinos et al., 2012
	Taiwanese cohort (n = 1272)	CV and all-cause mortality	Central systolic BP and PP	–	–	–	Wang et al., 2009
	Meta-analysis (n = 5648)	CV events	AIx, central PP	–	–	–	Vlachopoulos et al., 2010
Endothelial function							
Flow mediated dilation	Meta-analysis (n = 14,753)	CV events	FMD	–	–	–	Ras et al., 2013
	Multiethnic Study of Atherosclerosis (n = 1330)[c]	Incident CHD, CVD	FMD	Non-significant for both CHD and CVD	2.4% (CHD), 2.3% (CVD)	2.4% (CHD), 2.3% (CVD)	Yeboah et al., 2012
	Meta-analysis (n = 5547)	CV events	FMD	–	–	–	Inada et al., 2012
	Multiethnic Study of Atherosclerosis (n = 2843)	Incident CV events	FMD	– Non-significant	29%	– 28%	Yeboah et al., 2009
Endothelial peripheral arterial tonometry	Low-risk outpatients with chest pain (n = 270)	CV events	Log (RHI)	–	–	–	Rubinshtein et al., 2010
Circulating biomarkers related to vascular wall biology							
High sensitivity C- reactive protein	Emerging Risk Factors Collaboration meta-analysis (n = 166,596)	First CV event	Log (CRP), total and HDL cholesterol	0.0039	1.52%	–	Emerging Risk Factors Collaboration 2012
	Multiethnic Study of Atherosclerosis (n = 1330)[c]	Incident CHD, CVD	hsCRP	0.623 → 0.640(CHD), 0.623 → 0.640 (CVD)	7.9% (CHD), 3.7% (CVD)	7.9% (CHD), 3.7% (CVD)	Yeboah et al., 2012

ABI: ankle-brachial index; AIx: augmentation index; CHD, coronary heart disease; cIMT: carotid intima-media thickness; CV: cardiovascular; CVD, cardiovascular disease; FMD: flow mediated dilation; HDL: high density cholesterol; hsCRP: high sensitivity C-reactive protein; MI: myocardial infarction; PP: pulse pressure; PPA: pulse pressure amplification; PWV: pulse wave velocity; RHI: reactive hyperemia index; RM: reflection magnitude; SD: standard deviation.

[a] Change in C-statistic from addition of the novel marker to a classical risk factor model.

[b] Net reclassification index calculated only for individuals at intermediate risk according to the Framingham Risk Score.

[c] Only individuals at intermediate risk for cardiovascular events, according to the Framingham Risk Score, were included from the original cohort; in this case the overall and clinical net reclassification index are the same.

measurement of carotid plaque are available to date.

7. *Ease of use:* Experienced sonographers are required for reliable image acquisition, especially for plaque detection. Automated systems have facilitated cIMT measurements and 3D ultrasound may help to quantify atherosclerotic plaque volume and characteristics.

8. *Methodological consensus:* Strict standardization of ultrasound and reading protocols is required; the existing Mannheim consensus [9], American Society of Echocardiography consensus statement [10], and Prog-IMT procedures [32] are steps towards standardization.

9. *Reference values*:

cIMT: Reference values for common cIMT measured with the echotracking system have been reported [47].

Carotid plaque: Reference values for carotid plaque number, volume and other characteristics are still lacking. It is still unclear if the normal value should be "no carotid plaque", as plaques are frequently present in the elderly [25]. The concept of carotid plaque burden, based on a 3D ultrasound approach, may constitute a better metric of subclinical atherosclerosis [48–50].

Taken together, **cIMT** meets some of the 9 essential criteria to classify as a clinical surrogate endpoint, whereas **carotid plaque** meets most of them (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

Carotid ultrasound allows the simultaneous assessment of both cIMT and plaques. cIMT is validated by a substantial number of prospective long-term outcome studies. These have been performed in the general population, with sufficient number of events (myocardial infarction and stroke) and have included individuals at risk from various ethnic groups. Carotid plaque has incremental value to cIMT in risk assessment. Carotid ultrasound is relatively easy to use and can be applied to daily practice given the widespread availability of ultrasound devices [51].

The main disadvantage of cIMT is its low, yet significant, independent predictive power for future CV events over established risk scores. Increased cIMT values may not be solely attributed to atheromatosis of the intima; hypertension results in thickening of the media and, thus, in thicker cIMT. Nevertheless, the combined assessment of cIMT and plaque presence improves risk prediction. The meta-analyses suggest that cIMT measurements should not be routinely performed in the general population. The initial cost of the ultrasound device, probe and software, as well as training of sonographers should also be considered. Methodological issues pertaining to the site of measurements impede comparisons of cIMT values from different laboratories. Structured training and certification of sonographers should be sought, as well as certification for centres performing cIMT measurements by reference centres. The calibration of different ultrasound devices on the basis of a common phantom would be an additional step towards standardization; this approach has already been used for strain analysis in echocardiography ("The Leuven approach"). The cost-effectiveness of cIMT measurements and concomitant treatment should be assessed in depth. Regarding plaques, these are mainly prevalent in the elderly, which limits or even excludes their assessment in younger subjects below the age of 40–50 years. Currently, the low availability of 3D ultrasound, which can quantify total plaque volume and other plaque characteristics hampers detailed plaque characterization.

Current status in clinical practice guidelines:

The European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines for the management of hypertension have endorsed ultrasound scanning of the carotid arteries for cIMT measurement and plaque detection [23]; this is also the case in the ESC guidelines for CV disease prevention in individuals at intermediate risk (class IIa/B recommendation in both guidelines) [24]. The American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guidelines propose a IIa/B recommendation for risk assessment in individuals with intermediate risk (10-year risk for CV events of 6–20%) [52]. The most recent ACC/AHA guidelines for the assessment of CV risk in asymptomatic adults have given cIMT a class III/B recommendation for predicting a first atherosclerotic CV disease event; plaque assessment was not reviewed in these guidelines [3] (Table 6).

3. Ankle-brachial index (ABI)

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **IIa/A**.

In healthy individuals, systolic BP levels are physiologically higher in the lower extremities as compared to the arms; this is a combined effect of pulse wave reflections and amplification, as well as changes in vessel wall thickness attributed to hydrostatic pressure. This relationship can be quantified by the ratio of ankle-to-brachial systolic pressure, termed ABI; a decrease of this ratio heralds a late stage of atherosclerosis with hemodynamic compromise attributed to obstructive lesions. First described as a non-invasive method to diagnose lower extremity peripheral artery disease (PAD) [53], ABI has been subsequently scrutinized as a marker of CV disease prognosis, once the high risk of mortality and morbidity of patients with PAD was evidenced [54].

Methodology:

Its mode of measurement and calculation has recently been harmonized [55]. The standard mode of measurement requires a (handheld) continuous wave Doppler device and a manual blood pressure (BP) cuff. From this respect, it is one of the less expensive and most available methods to detect atherosclerosis and stratify CV risk. While ABI is traditionally calculated at each ankle by taking the highest pressure between the systolic BPs of the posterior tibial and dorsalis pedis artery divided by the highest systolic BP between both arms, the lower of the 2 lower limbs ABI is retained to assess CV risk of the subject [55]. In healthy subjects at low risk for PAD, trivial intrinsic differences of ABI values have been reported between men and women, and in subjects of African descent versus other ethnic groups [56]. While these small differences (<0.02) may impact the estimation of PAD in these different groups, they are not clinically relevant at an individual level, so similar normal values are accepted.

The ABI threshold to diagnose PAD is commonly at 0.90. At higher levels (>1.40) ABI identifies medial calcinosis, i.e. calcification of the tunica media without affecting the arterial lumen, a condition distinct from atherosclerosis. Stiffened arteries due to this disease are mostly observed in aged population, especially in case of diabetes and/or end-stage renal failure.

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* This is intuitive, as PAD (i.e. a low ABI) is causally linked to CV risk factors (Table 2).

2. *Prospective validation and* 3. *Incremental value:* Several longitudinal studies in the general population have demonstrated the predictive ability of ABI for overall and CV mortality and morbidity (Table 3). In an individual-based meta-analysis collecting data of >48,000 men and women through 16 studies, it has been demonstrated that an ABI <0.90 is associated with increased risk of mortality (hazard ratio [HR]: 3.33 in men and 2.71 in women) [57]. Actually the risk of mortality (or CV events) increases when ABI values fall below 1.10, but becomes more significant and consistent below 0.90. Subjects with an ABI between 1.10 and 1.40 are at the lowest risk levels of CV events. In case of high (>1.40) ABI, the risk of total and CV mortality is also increased [57]. However, in this case, the intra-arterial pressure of leg arteries cannot be accurately estimated by the cuff. In approximately half of these cases,

Table 4
Assessment of vascular biomarkers.

	Proof of concept	Prospective validation	Incremental value	Clinical utility	Clinical outcomes	Cost-effectiveness	Ease of use	Methodological consensus	Reference values
Carotid ultrasonography	++++	+++	+++	++	+/−	+	++	++	Yes, for cIMT measured with the echotracking method.
Ankle-brachial index	++++	++++	+++	+++	+/−	−	++++	++++	Diagnostic thresholds for PAD (cutoff value: 0.90)
Arterial stiffness									
Carotid-femoral pulse wave velocity	++++	++++	++++	+++	+	−	+++	+++	Yes
Brachial-ankle pulse wave velocity	++++	+++	++	+	−	−	++++	+++	Yes, for Asian populations.
Central haemodynamics/ Wave reflections	++++	+++	+++	++	+	−	+++	+++	Yes
Endothelial function									
Flow mediated dilatation	++++	+++	+	+	+/−	−	+	++	No
Endothelial peripheral arterial tonometry	+++	++	+	−	−	−	+++	+	No
Circulating biomarkers related to vascular wall biology									
High sensitivity C-reactive protein	+++	+++	++	+++	++	+	++++	+++	Cutoff value: 2 mg/L.

occlusive artery disease due to atherosclerosis coexists [58]. Two studies support the hypothesis that in patients with very high ABI, only those with concomitant medial calcinosis and occlusive disease are at increased risk of death [59,60]. Hence, in case of an ABI >1.40, further tests (e.g. toe-brachial index, or Doppler flow analysis) is mandatory to refine the risk estimation.

4. Clinical utility: Owing to its diagnostic role for PAD (values <0.9 or >1.4), ABI can change predicted risk sufficiently to change recommended therapy. The ARIC study reported modest risk prediction improvement with use of ABI in addition to the FRS; this needs further evaluation, especially because ABI was measured only in one leg, so that it is estimated that 1/4th of PAD cases were not detected [61]. In addition, the oscillometric method was used to determine limb pressures [55,62]. The validity of this method to estimate accurately ankle artery pressure is still a matter of controversy, since it presents uncertain validity and reproducibility in case of PAD [54,55].

The ABI collaboration meta-analysis showed that 19% of men and 36% of women can have their risk estimation reclassified if the ABI is performed in addition to the FRS [57]. In predicting events, the C-index increased modestly in men, from 0.672 when using the FRS alone to 0.685 when adding ABI to FRS, and improved dramatically in women, from 0.578 to 0.690, with NRI of 4.3% in men and 9.6% in women. Restricting the analysis in those at intermediate 10-year risk of 10–19% resulted in higher NRIs, both in men (15.9%) and women (23.3%) [63]. In an intermediate-risk subgroup from the Framingham Offspring Study, an NRI of 7.9% for coronary heart disease was reported [64] (Table 3).

5. Clinical outcomes: Whether the use of ABI can ultimately lead to improved prognosis after prompt intervention requires further investigation in randomized controlled trials. One open trial comparing aspirin 100 mg vs. placebo in patients with ABI <0.90 detected after a large population screening failed to show any benefit after 8 years of follow-up [65]. In another double-blinded trial limited to diabetic patients with ABI <1.0, aspirin 100 mg was not superior to placebo to decrease mortality or CV events [66]. However, an analysis for the NHANES cohort [67] study suggests better outcome in individuals with an ABI <0.90 who were at least on two out of three major CV protective drugs (i.e. antiplatelet agents, angiotensin-converting enzyme inhibitors [ACE-Is]/

Table 5
Usefulness of vascular biomarkers for primary and secondary CVD prevention.

	Recommendation	Level of evidence	Comments
Carotid ultrasonography	IIa	A	Moderate usefulness for risk stratification. Concomitant identification of plaque presence.
Ankle-brachial index	IIa	A	Useful for risk stratification, especially women.
Arterial stiffness			
Carotid-femoral pulse wave velocity	IIa	A	Useful for risk stratification.
Brachial-ankle pulse wave velocity	IIb	B	
Central haemodynamics/Wave reflections	IIb	B	
Endothelial function			
Flow mediated dilatation	III	B	Requires skilled, trained operator. Reactive hyperaemia is stressful. Methodological problems are not resolved. Added value is not proven.
Endothelial peripheral arterial tonometry	III	C	Reactive hyperaemia is stressful. Added value is not proven.
Circulating biomarkers related to vascular wall biology			
High sensitivity C- reactive protein	IIb	B	

Table 6
Implementation of vascular biomarkers in guidelines.

	ESC/EASD guidelines on diabetes, pre-diabetes and cardiovascular diseases (2013)	ESC/ESH guidelines for the management of arterial hypertension (2013)	ESC guidelines on cardiovascular disease prevention in clinical practice (2012)	ESC/EAS guidelines for the management of dyslipidaemias (2011)	ACC/AHA guideline on the assessment of cardiovascular risk (2013)	ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults (2013)	ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults (2010)
Carotid ultrasonography	May be considered as useful cardiovascular marker, adding predictive value to the usual risk estimate.	Class IIa/level of evidence: B	- Asymptomatic adults at moderate risk: Class IIa/level of evidence: B	It is indicated to screen for dyslipidaemias in the presence of increased cIMT or carotid plaques.	Class III/level of evidence: B	–	Class IIa/level of evidence: B
Ankle-brachial index	May be considered as useful cardiovascular marker, adding predictive value to the usual risk estimate.	Class IIa/level of evidence: B		PAD is a high risk condition, and lipid-lowering therapy (mostly statins) is recommended: Class I/level of evidence: A	Class IIb/level of evidence: B	–	Class IIa/level of evidence: B
Arterial stiffness							
Carotid-femoral pulse wave velocity	May be considered as useful cardiovascular markers, adding predictive value to the usual risk estimate.	Class IIa/level of evidence: B	–	–	–	–	Class III/level of evidence: C
Brachial-ankle pulse wave velocity	May be considered as useful cardiovascular markers, adding predictive value to the usual risk estimate.	–	–	–	–	–	Class III/level of evidence: C
Central haemodynamics/ Wave reflections	–	–	–	–	–	–	–
Endothelial function							
Flow mediated dilation	–	–	–	–	–	–	Class III/level of evidence: B
Endothelial peripheral arterial tonometry	–	–	–	–	–	–	–
Circulating biomarkers related to vascular wall biology							
High sensitivity C- reactive protein	–	–	- As part of refined risk assessment in patients with an unusual or moderate CVD risk profile: Class IIb/level of evidence: B - Asymptomatic low-risk individuals and high-risk patients: Class III/level of evidence: B	- Individuals with increased hsCRP have a higher risk level than that calculated from the SCORE chart - Not recommended as a secondary target of therapy for everybody; however, it may be useful in people close to the high risk category to better stratify their total CV risk.	Class IIb/level of evidence: B	High sensitivity-CRP level >2 mg/L is an additional factor to be considered during treatment decision making for individuals without atherosclerotic disease	- Men ≥50 years old or women ≥60 years old with low-density lipoprotein cholesterol less than 130 mg/dL; not on lipid-lowering, hormone replacement, or immunosuppressant therapy; without clinical CHD, diabetes, chronic kidney disease, severe inflammatory conditions, or contraindications to statins: Class IIa/level of evidence: B - Asymptomatic intermediate-risk men ≤50 years old or women ≤60 years old: Class IIb/level of evidence: B - Asymptomatic high-risk adults: Class III/level of evidence: B

angiotensin receptor blockers and/or statins), but this merits confirmation with an appropriately designed trial. A meta-analysis (n = 11,686) reported that various drug classes improve walking distance in patients with an ABI<0.90, albeit with limited benefits; statins were the most efficient among them [68].

Apart from single measurements, ABI change over time can also provide predictive information (except when revascularization is performed, which usually leads to an ABI increase when successful). An ABI decrease of more than 0.15 within 3 years was associated with a 2.4-fold risk increase of death, and 2.8-fold risk increase of CV mortality [69]. With respect to its ability to monitor and guide therapy, intra- and inter-observer reproducibility is acceptable (at best ±0.10) but not sufficient to detect individual small values change during follow-up [55]. At population level, ABI changes slowly over time, so that this marker is not highly sensitive to risk factor modification or pharmacological intervention.

6. *Cost-effectiveness:* At present, no data regarding cost-effectiveness have been published. Nonetheless, the initial cost of the equipment is relatively low.

7. *Ease of use:* ABI can be easily measured after an initial training regarding the use of the Doppler device.

8. *Methodological consensus:* The measurement and interpretation of the ABI has been standardized [55].

9. *Reference values:* Cutoff points for PAD have been ascertained, as mentioned above. The same cutoff points (values <0.90 or >1.40) are used to detect individuals at increased risk for CV events independently of the presence of symptoms of PAD and other risk factors [55,57].

In summary, ABI meets most of the 9 essential criteria to classify as a clinical surrogate endpoint (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

A unique advantage is that ABI is the only biomarker that has both a diagnostic (for PAD) and prognostic (for CV disease and mortality) role. An additional advantage is its wide availability, so that it can currently be considered as the first-line vascular biomarker to be used in the setting of primary care; however, skilled operators are required for consistent, accurate results. The low cost of the equipment and the extensive validation are also ranked among the strengths of the method.

The disadvantages of the ABI include false negative results in patients with medial calcinosis because stiff arteries produce falsely elevated ankle pressure (see above). Resting ABI is insensitive to mild PAD; treadmill tests are sometimes used to increase sensitivity, but this increases assessment duration and is unsuitable for patients who are obese or have co-morbidities. Measurement of the ABI can be time-consuming in the setting of PAD.

A number of issues are open. Devices using non-Doppler based methodology should be accredited. Further research should explore potentially easier and faster alternative methods for ABI measurement that would likely be implemented more broadly in primary care. Finally, the optimal method of ABI calculation for predicting CV events and PAD merits additional investigation [55].

Current status in clinical practice guidelines:

The ESH/ESC guidelines for the management of hypertension state that ABI should be considered for detecting PAD in hypertensives (class IIa/B recommendation) [23]; the same holds true in the ESC guidelines for CV disease prevention in individuals at intermediate risk (class IIa/B recommendation) [24]. The European Society of Cardiology/European Association for the Study of Diabetes (ESC/EASD) guidelines for diabetes and pre-diabetes state that ABI may be considered as a useful marker that adds predictive value to the usual risk estimate [70]. The U.S. Preventive Services Task Force concluded that the current evidence is insufficient to assess the balance of benefits and harms of screening for PAD and CVD risk assessment with the ABI [71]. According to the ACCF/AHA guidelines, measurement of ABI is reasonable for the assessment of CV risk in asymptomatic adults (class IIa/B recommendation) [52]. The most recent ACC/AHA guidelines state that ABI measurement may be considered if, after quantitative risk assessment, a risk-based treatment decision is uncertain (class IIb/B recommendation) [3] (Table 6).

4. Arterial stiffness

Arterial stiffening results primarily from arteriosclerosis (principally a disease of the media, related to normal or accelerated aging) rather than from atherosclerosis (principally a disease of the intima, affecting the vessel in a patchy and not uniform manner). Because waves travel faster in a rigid tube, loss of compliance results in increased velocity of pulse waves; therefore, a high pulse wave velocity is a hallmark of arteriosclerosis. CV risk factors alter the composition and mechanical properties of arterial walls making them eventually less compliant. Elastic-type arteries, such as the aorta, are primarily affected, as opposed to muscular arteries.

A multitude of invasive and non-invasive methods measuring arterial stiffness have been described. The most widely used and validated techniques involve the assessment of pulse waves as they travel over a significant portion of the arterial tree; regional (over shorter arterial segments) and local (carotid or femoral) arterial stiffness are emerging as promising biomarkers [72]. Carotid-femoral pulse wave velocity (cfPWV) and brachial-ankle PWV (baPWV) are dealt with herein. Cardio-ankle vascular index (CAVI) has been recently introduced. CAVI is primarily used in Japan and has the theoretical advantage to be less dependent on BP levels. CAVI had been correlated with several arteriosclerotic and atherosclerotic diseases [73].

4.1. Carotid-femoral pulse wave velocity (cfPWV)

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **IIa/A**.

cfPWV, i.e. the velocity of the pulse as it travels from the heart to the carotid and the femoral artery, remains the most commonly used non-invasive method and is considered as the “gold standard” [74]. Data regarding risk prediction are less consistent concerning local stiffness, i.e. carotid stiffness measured with high-resolution echotracking systems, or arterial stiffness measured at other arterial sites such as carotid-radial PWV and femoro-tibial PWV.

Methodology:

cfPWV is usually measured using the “foot-to-foot” velocity method from a number of waveforms. These are usually obtained using surface tonometry probes at the right common carotid artery and the right femoral artery and the time delay (Dt, or transit time) is measured between the “foot” of the two waveforms [75]. The “foot” of the wave is defined at the end of diastole, when the steep rise of the wavefront begins. The transit time is the time of travel of the “foot” of the wave over a known distance. A variety of different waveforms can be used including pressure [75], distension [76] and flow [77]. The distance D covered by the waves is usually assimilated to the skin distance between the two recording sites, i.e. the common carotid artery and the common femoral artery. PWV is calculated as PWV = D/Dt (m/s).

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* A large number of studies have reported the various physiological and pathophysiological conditions associated with increased arterial stiffness [74]. Apart from the dominant effect of BP and aging, these include genetic background, CV risk factors and diseases, and also primarily non-CV diseases (such as end-stage renal disease [78]) and chronic inflammatory diseases (such as inflammatory bowel disease [79]). Arterial stiffness is a

cumulative measure of their damaging effects on the arterial wall (Table 2).

2. *Prospective validation and* 3. *Incremental value:* The predictive value of arterial stiffness has been largely demonstrated. Currently, numerous studies in patients with uncomplicated essential hypertension [80–82], type 2 diabetes mellitus [77], end-stage renal disease [83], elderly subjects [84,85] and the general population have prospectively validated cfPWV [86]. The independent predictive value of arterial stiffness has also been demonstrated for neurologic functional outcome after stroke [87]. Arterial stiffness is a robust predictor of all-cause and CV mortality, fatal and non-fatal coronary events and fatal strokes [88,89], thus it can be considered as an intermediate endpoint for CV events [90]. In an individual data meta-analysis, CV disease events increased by 30% per 1-standard deviation (SD) increase of cfPWV (95% CI: 1.18–1.43) after adjustment for traditional risk factors [88]. The independent association with all-cause mortality merits attention, as it indicates that the role of arterial stiffness extends beyond diseases of the CV system. Moreover, it should be noted that the independent predictive value of arterial stiffness has been demonstrated after adjustment for classical CV risk factors, including brachial pulse pressure. In addition, arterial stiffness retains its predictive value for CV events after adjustment for the FRS [80] or SCORE [91], suggesting that it has an added value to a combination of CV risk factors (Table 3).

4. *Clinical utility:* Two studies and an individual data meta-analysis showed that patients at intermediate risk could be reclassified into a higher or lower CV risk category when arterial stiffness was measured [86,91,92]. Specifically, in the Framingham study 15.7% of patients at intermediate risk could be reclassified into a higher (14.3%) or lower (1.4%) risk category [92]. In a recently published individual data meta-analysis (n = 17,635 participants), the 5-year overall NRI for coronary heart disease and stroke in intermediate risk individuals was 14.8% and 19.2% respectively [88]. Finally, 29% of patients with chronic kidney disease were reclassified into lower or higher risk for all-cause mortality when arterial stiffness was taken in to account [93] (Table 3).

5. *Clinical outcomes:* No study or randomized controlled trial has yet assessed the potential of cfPWV as a target for therapy and whether such a strategy would result in better clinical outcomes. One study in end-stage renal disease patients showed a better outcome for those that decreased their arterial stiffness [94]; this remains to be validated in a larger population of patients at lower CV risk. Such a study is currently underway [90].

6. *Cost-effectiveness:* No data are currently available. Wide adoption of the technique leads to continuous reduction of device cost; the potential for cost saving is high given the low cost of individual examination and high reclassification.

7. *Ease of use:* Accurate recording of carotid and femoral pulses is easily performed after a short learning period; exposure of the inguinal region is, however, a drawback.

8. *Methodological consensus:* An expert consensus document for the measurement of cfPWV in daily practice has been published [76].

9. *Reference values:* Reference values for PWV have been established in 1455 healthy subjects and a larger population of 11,092 subjects with CV risk factors [95]. These are reported according to age and BP levels. Although the relationship between cfPWV and CV events is continuous, a threshold of 12 m/s had been initially suggested as a conservative estimate of significant alterations of aortic function in middle aged hypertensives [74], and included in the ESH/ESC guidelines for the management of hypertension. This threshold was based on cfPWV using the full direct carotid-to-femoral distance and has been revised in a recent consensus document to 10 m/s [76], in order to normalise PWV values according to the arterial pathway. Accordingly, the investigator should preferentially use the direct carotid-femoral distance and multiply by 0.8 only marginally overestimating the real travelled distance by 0.4% [76,96]. The new threshold is included in the 2013 ESH/ESC guidelines for the management of hypertension [23].

In sum, cfPWV meets most of the 9 essential criteria to classify as a clinical surrogate endpoint (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

Aortic stiffness, as measured by cfPWV, integrates the damage of risk factors on the aortic wall over a long period, whereas BP, glycaemia, and lipids can fluctuate and their values, recorded at the time of risk assessment, may not reflect the average values damaging the arterial wall. Nevertheless, cfPWV is strongly influenced by age and BP levels, while the influence of other risk factors is weaker [97]. Its non-invasive nature, fast learning curve, the wide choice of available devices/no absolute need for a proprietary device, relatively low cost, extensive validation in large population studies and established reference values are among its advantages. On the other hand, exposure of the inguinal region for measurements and a calibration of the measured travelled distance are needed.

cfPWV meets most of the criteria to qualify as a surrogate endpoint for CV disease. Currently, studies that address its ability to monitor and guide therapy and eventually improve outcomes are in progress; though it cannot be considered yet as a surrogate endpoint, it is at an advanced stage of validation.

Current status in clinical practice guidelines:

At present, the ESC guidelines for individuals at intermediate risk acknowledge the added value of cfPWV for the stratification of patients [24]. Moreover, it should be considered for hypertensives (class IIa/B recommendation) [23], and it can add predictive value to the usual risk estimate of diabetics [70]. In contrast, it is not recommended by the ACCF/AHA guidelines for the assessment of CV risk in asymptomatic adults (class III/B recommendation) [52]; nevertheless, these need to be reappraised in the light of recent data [88]. (Table 6)

4.2. Brachial-ankle pulse wave velocity (baPWV)

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **IIb/B**.

baPWV is a method for assessing arterial stiffness. Though it shares the same theoretical background with cfPWV, baPWV capitalizes on the concept that measurements over a longer arterial length may provide additional information and be easier, as it only involves wrapping of a pressure cuff in each of the four exposed extremities [98]. The method has is being primarily used in Japan.

Methodology:

baPWV is measured using a volume-plethysmographic apparatus. Occlusion cuffs, connected to both plethysmographic and oscillometric sensors, are wrapped around both upper arms and ankles of the subjects lying in the supine position. The brachial and posterior tibial arterial pressure waveforms are recorded by the plethysmographic sensor. The path lengths from the suprasternal notch to the brachium (Lb) and from the suprasternal notch to the ankle (La) are obtained from superficial measurements and corrected for the height of the individual using validated equations. baPWV is then calculated according to the equation: $baPWV = (La-Lb)/\Delta Tba$, where ΔTba is the time interval between the wavefront of the brachial waveform and that of the ankle waveform [99].

Considering the results of published prospective studies, a baPWV value of 18 m/s may be used as a cutoff value in the assessment of the risk for CV disease [100,101]. Due to the fact that

age and BP are major determinants of baPWV, a nomogram describing the correlation of the three variables is available [102].

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* baPWV has been well validated as a CV risk marker, as it is closely correlated not only with cfPWV, but also with aortic PWV assessed by the invasive method [98,103]. Similarly to cfPWV, the presence of CV risk factors is linked to elevated baPWV values [104] (Table 2).

2. *Prospective validation and* 3. *Incremental value:* For primary prevention, several prospective studies have reported that baPWV may be a useful predictor of future CV events in patients with end-stage renal disease, hypertension and in the general population [100,105]. For secondary prevention, baPWV has been suggested as a useful predictor of the prognosis in patients with acute coronary syndromes and heart failure [101,106]. Some prospective studies also demonstrated that an elevated baPWV is a predictor of progression of pathophysiological abnormalities in the early stages of hypertension and chronic kidney disease [103]. A meta-analysis demonstrated that an increase in baPWV by 1 m/s was associated with an increase by 12%, 13% and 6% in CV events, CV mortality, and all-cause mortality, respectively; the *independent* predictive value of baPWV was established when studies that had controlled for most CV risk factors were subsequently analyzed [107]. The potential clinical advantage of baPWV over traditional risk scores has not been, however, formally proven (Table 3).

4. *Clinical utility*: No data exist regarding the potential of the method for reclassification and subsequent implications for therapy. Despite that, an improvement in baPWV following drug therapy for hypertension, dyslipidaemia and diabetes mellitus and lifestyle modifications (exercise, weight reduction, smoking cessation) has been shown [108].

5. Clinical outcomes: No studies exist to date to substantiate the prospect that treatment according to baPWV values can lead to better clinical outcomes.

6. *Cost-effectiveness:* The cost-effectiveness of this method has not been addressed yet.

7. *Ease of use:* The technique is easy to use, as measurements are automatically performed after cuff placement at the extremities.

8. *Methodological consensus:* Measurements are performed according to manufacturer's instructions; however, a consensus paper has not been published.

9. *Reference values:* Reference values have been published for Chinese populations [109,110]; no data exist for non-Asian populations. Similar to cfPWV, these are reported according to age and BP levels.

In conclusion, baPWV meets some of the 9 essential criteria in order to be considered a clinical surrogate endpoint (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

Simplicity and concurrent ABI measurement (albeit with the oscillometric method; see section 3. ABI) with the same device are considerable advantages. The disadvantages of the method include issues pertaining to calculation of travelled distance (height-based formulas), need for validation in diverse populations and lack of reference values. Importantly, applicability may be limited when arteries of smaller calibre are affected, such as in diabetes mellitus or in distal PAD.

It should be emphasized that cfPWV data (which assess predominantly elastic type arteries) are not automatically extrapolated to baPWV, which reflects the stiffness of the large-to middle-sized arteries, and therefore, elastic arteries are assessed along with muscular and mixed-type arteries [98].

Comparisons with the "gold standard" method of cfPWV are needed. Despite the fact that the incremental value of baPWV over and above traditional risk factors has been demonstrated in high-risk populations, prospective studies are needed to examine its incremental value over risk scores in the general population. Moreover, it is currently unknown whether improvement of baPWV with interventions might also translate into successful prevention of CV events.

Current status in clinical practice guidelines:

At present, baPWV has not been endorsed by guidelines (Table 6).

5. Central haemodynamics/wave reflections

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **IIb/B**.

Left ventricular afterload is dependent on aortic valve and peripheral circulation properties (steady-state component) and on the elasticity of the aorta and the large arteries (pulsatile component). Different models allow quantification of systemic circulation mechanics. In the most realistic ones, pressure and flow waves are generated with each heartbeat and are propagated towards the periphery where they are reflected backwards (towards the heart) for various reasons (stiffness gradient, presence of bifurcations, abrupt diameter gradient in arterioles). On their return, the reflected waves merge with the antegrade wave and amplify it [111]. As a result of this (but not exclusively), peripheral BPs are higher compared to central (aortic) BPs to a varying degree, a phenomenon named "amplification" [112]. Central BPs are more relevant than peripheral ones, as the heart, brain and kidneys are directly exposed to them [113]. With aging, the arrival of reflected waves in the ascending aorta is shifted into systole due to earlier wave return; this is largely attributed to increased aortic stiffness/PWV and the resulting wave changes shape. With vasoconstriction, the amplitude of the reflected pressure waves increases. Both these processes lead to increased central systolic pressure (increased cardiac load and oxygen consumption), lower diastolic pressure (decreased myocardial perfusion pressure) and promotion of degeneration of the elastic components of the arterial wall. The net result is an imbalance towards myocardial ischaemia and an impairment of (mainly diastolic) left ventricular function. Of note, in the presence of systolic heart failure the forward wave is of lesser magnitude and as a result, wave reflections are reduced.

Methodology:

Central haemodynamic indices are either central BP parameters and derivatives (central systolic BP, pulse pressure, augmented pressure and amplification) or indices that quantify wave reflections [augmentation index (AIx), forward and backward wave, wave intensity analysis]. Invasive recordings provide accurate measurements of central pressures and wave reflection. In clinical studies, however, non-invasive methods are used for quantification. *Classical pulse wave analysis* [111] involves analysis of pressure waves alone, from the carotid, radial, or brachial artery. The subsequent use of a transfer function (or special algorithms) and calibration to non-invasively measured pressure yields parameters of the central waveforms, such as central (aortic) pressure, augmentation pressure (measure of the enhancement of central aortic pressure by the reflected pulse wave) and AIx (augmentation pressure to pulse pressure ratio). From pressure waves and simultaneously acquired or estimated flow waves [114], *wave separation analysis* (assumes a set forward wave, a limitation of the approach) has been used to acquire amplitudes of forward (Pf) and backward (Pb) waves and their ratio, termed reflection magnitude. It should be emphasized that indices and their individual validation for clinical use are not interchangeable; for example, augmented pressure and reflection magnitude have a better predictive ability than the AIx [115,116].

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* Increased wave reflection is related to the extent of myocardial ischaemia in patients with [117] and without [118] obstructive coronary artery disease. Moreover, it is directly related to left ventricular hypertrophy [119] and its regression with treatment [120], to left atrial size [121], and is inversely related to left ventricular diastolic function at rest [121] and during exercise [122]. Measures of wave reflection can improve the diagnostic accuracy in patients with suspected heart failure with preserved ejection fraction [121]. An increase in wave reflection has been associated with a decreased renal function [123] and with an impaired outcome following acute ischemic stroke [87] (Table 2).

2. *Prospective validation and* 3. *Incremental value:* Measures of wave reflection have consistently been reported to be independent predictors of either CV events or CV mortality in high risk populations: patients with impaired renal function [124], renal transplant recipients [115], dialysis patients [78], patients undergoing coronary interventions [125], patients undergoing coronary angiography [126], and patients hospitalized due to acute heart failure [127]. Results are less consistent in broader populations: in most [116,128], but not all [92] community-based studies an independent predictive value of measures of wave reflections has been shown. Results are heterogeneous in studies on hypertensive patients [129] and in geriatric patients [130]. In almost all studies, the predictive value was additional to that of brachial BP.

In a meta-analysis of 5648 subjects, central systolic BP, central pulse pressure and central AIx were independent predictors of CV events; of interest, central AIx also independently predicted all-cause mortality, a finding that extends its role beyond the CV system. Central pulse pressure had a marginally significant ($P = 0.057$) better predictive ability for clinical events when compared with peripheral pulse pressure [131]; when one study with methodological shortcomings was excluded, statistical significance was reached [132,133] (Table 3).

4. *Clinical utility:* In the largest population study, wave reflections (expressed as reflection magnitude) predicted the occurrence of heart failure and severe CV events [116]. Several novel indices of clinical utility and reclassification were computed, showing an NRI of 13% for hard CV events and of 38% for heart failure, as compared to models that included brachial systolic and diastolic BP among other traditional risk factors (Table 3).

5. Clinical outcomes: Antihypertensive drug trials have demonstrated an improvement in intermediate endpoints, such as left ventricular mass, following a reduction of wave reflections and central pressures [134,135]. Evidence showing that an improvement in wave reflection will lead to a reduction in CV events was provided by the Conduit Artery Functional Evaluation (CAFÉ) study, where, despite a similar reduction in peripheral systolic BP, a calcium-channel blocker regimen was more effective in lowering central systolic BP, and reduced future CV events compared to a beta-blocker regimen [129].

6. *Cost-effectiveness:* No studies so far have investigated the cost effectiveness of measures of central haemodynamics/wave reflections. Nevertheless, the use of central BPs, compared to peripheral BPs, for guiding antihypertensive treatment led to less use of medications [136]. The assessment of central haemodynamics is valuable in the evaluation of young persons with isolated systolic hypertension by identifying those who do not have concurrently increased central pressures, a common pattern, thus obviating the need for further investigations and drug treatment [23,137].

7. *Ease of use:* Assessment of wave reflections/central BPs with peripheral tonometry can be made by validated, non-invasive devices. Cuff-based techniques are more easy to use and offer simultaneous assessment of both brachial and aortic BP, as well as the opportunity of 24-h recordings; they predict left ventricular hypertrophy more accurately than peripheral ambulatory blood pressure monitoring [138]. Nevertheless, the technical validation of these latter devices is ongoing and needs to be done at rest and with exercise [139,140].

8. *Methodological consensus:* It is yet unclear which index will prove to be the most useful for clinical practice and how this should be measured. A consensus statement on central BP measurements and antihypertensive therapy has been published; an updated version is warranted [141].

9. *Reference values:* Reference values for central haemodynamics have been recently published [142].

As a result, wave reflections and central haemodynamics meet some of the 9 essential criteria to classify as a clinical surrogate endpoint (Tables 4 and 5)

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

Advantages include the strong physiological background and the clinical value in high-risk patients. The incremental value of wave reflections/central haemodynamics has been shown to some degree; however, more data are needed regarding clinical utility, improvement of clinical outcomes and cost-effectiveness.

Because peripheral pressure is necessary to calibrate central pressure, their respective clinical values cannot be easily disentangled. It needs to be clarified, which is the ideal measure of wave reflections (*pulse wave analysis-based indices:* AIx, augmented pressure; *wave separation analysis-based indices:* amplitude of backward wave, reflection magnitude; *wave intensity analysis-based indices:* wave reflection index, intensity of forward compression and expansion waves) and central pressures. Simplifications of systems for assessment that facilitate adoption by clinicians are welcome.

Current status in clinical practice guidelines:

The ESH/ESC guidelines for the management of arterial hypertension state that central BPs/AIx can be helpful when assessing young patients with isolated systolic hypertension; however, more data are required before central haemodynamic indices are recommended for routine use in hypertensives in general [23] (Table 6).

6. Endothelial function

A multitude of techniques for assessing endothelial function have been described. The focus of this document is on non-invasive methods owing to their ease of use that permits implementation in clinical practice beyond vascular laboratories. Invasive methods and laboratory techniques requiring high levels of technical expertise remain outside the scope of this document and will not be addressed. Such methods (coronary endothelial function, venous occlusion plethysmography, pulse wave analysis for endothelial function assessment, laser Doppler flowmetry, biochemical markers and bioassays, endothelial microparticles, progenitor cells and glycocalyx) have been previously reviewed in a position statement by the ESC Working Group on Peripheral Circulation [143].

6.1. Flow-mediated dilation (FMD)

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **III/B**.

The endothelium regulates all aspects of vascular homeostasis by releasing vasoactive molecules in response to physical and chemical stimuli. Endothelium-derived nitric oxide (NO) is the main mediator of all vasoprotective effects; NO is a very potent vasodilator but also has anti-inflammatory, anti-proliferative and antithrombotic properties. Reduced NO bioavailability, due to reduced production and/or increased inactivation, results from

altered redox and inflammatory state. Endothelial dysfunction is present very early in the atherosclerotic process, long before structural changes in vessel wall are evident [144]. This dysfunction contributes to the development and progression of atherosclerosis and even plaque rupture [143].

Endothelial function was initially assessed using invasive methods and pharmacological stimuli in the coronary arteries and in the peripheral resistance arteries (venous occlusion plethysmography). Brachial artery FMD was the first non-invasive technique to assess endothelial function in the peripheral conduit arteries [145] and remains today the most widely applied method [143].

Methodology:

FMD is an endothelium-dependent, NO-mediated process that uses high-resolution ultrasound in the brachial artery by means of a high frequency linear transducer to monitor changes in arterial diameter in response to increased blood flow, an important physiological stimulus for endothelial NO production [146]. In brief, images are acquired at baseline and after deflation of a cuff inflated to at least 50 mmHg above systolic BP [146] or at about 250 mmHg [147] for a set length of time, usually 4–5 min. Placement of the cuff above or below the elbow has been the subject of controversy; upper versus lower cuff placement maximizes hyperaemic response but distorts the brachial artery and introduces variability in results. FMD is defined as the maximum percent increase in arterial end-diastolic diameter during the first minutes of hyperaemia compared with the diameter at rest [147]. To assess the endothelium-independent smooth muscle capacity to dilate, nitrate-mediated dilation is also determined as the percent increase in brachial artery diameter 4 min after administering sublingual glyceryl trinitrate. FMD has been closely correlated with endothelial function assessed in the coronary circulation [145,148]; variability across laboratories and reproducibility over the short and medium term are vey good [149].

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* Endothelial dysfunction, assessed by impaired FMD, has been extensively associated with most of the established and emerging CV risk factors (e.g. dyslipidaemia, hypertension, smoking, diabetes mellitus, family history of premature atherosclerosis, elevated plasma homocysteine), as well as with the presence and extent of structural arterial disease and prevalent CV disease. Assessment of endothelial dysfunction using FMD appears to complement other imaging endpoints of structural arterial disease burden, probably preceding their occurrence [150] (Table 2).

2. *Prospective validation and* 3. *Incremental value:* FMD has been prospectively validated in predicting future CV events. Its prognostic value has been demonstrated in patients with advanced atherosclerosis, in subjects at high CV risk and more recently also in low-risk subjects, although this was not a consistent finding in all studies. Three meta-analyses showed an independent prognostic value [151–153]; for each 1% increase in FMD, risk was decreased by 13% (relative risk [RR]: 0.87; 95% CI: 0.83–0.91), 8% (RR: 0.92; 95% CI: 0.88–0.95) and 10% (RR: 0.90; 95% CI: 0.88–0.92) respectively. The correlation of FMD with risk was stronger in populations with overt CV disease.

The incremental value of FMD in addition to classical CV risk factor evaluation using risk scores such as the SCORE and FRS has not been established. Few studies so far have reported on the incremental value of FMD using the change in the area under the curve with conflicting results [154]. (Table 3) Of note, invasive methods for assessment of endothelial function have shown incremental value over the FRS [155].

4. *Clinical utility:* The reclassification potential of FMD has been examined in the MESA cohort; in intermediate risk individuals the NRI was 2.4% for incident coronary heart disease [64]. (Table 3)

5. *Clinical outcomes:* Numerous studies have incorporated FMD measurements because it improves rapidly with physiological and pharmacological interventions (e.g. statins, ACE-Is, calcium channel blockers, exercise, weight loss, estrogen, antioxidants, vitamins, dietary constituents). This reinforces the concept that endothelial dysfunction, the earliest stage of atherosclerosis, is a reversible process [156,157]. Nevertheless, whether an improvement in FMD with treatment may also translate into improved clinical outcomes has not been tested in a randomized controlled trial. In non-randomized controlled studies, FMD improvement was associated with a reduction in events in hypertensive postmenopausal women [158], whereas persistent FMD impairment despite optimized therapy was associated with an increase in CV events [159,160].

6. *Cost-effectiveness:* The cost-effectiveness of FMD has not been studied so far.

7. *Ease of use:* FMD measurements require significant technical expertise; guidelines propose a minimum number of 100 supervised scans prior to scanning independently and at least 100 scans/year to maintain competency [146].

8. *Methodological consensus:* Published guidelines have facilitated uniform FMD measurements [146]. Nevertheless, methodological differences have been reported regarding the level of inflation pressure to cause ischaemia, duration of ischaemia, brachial versus wrist cuff inflation and the time point at which the effect of reactive hyperaemia is assessed.

9. *Reference values:* Currently there are no reference values for FMD.

In conclusion, FMD does not fulfill the 9 essential criteria in order to be considered a clinical surrogate endpoint (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

FMD is an integrative marker of the damage from risk factors on the arterial wall and a valuable research tool in the study of the role of risk factors in atherosclerosis [146]. The most important role of FMD appears to be its ability to monitor the effect of a treatment or an intervention on endothelial function. Its rapid response to treatment stirred the anticipation that it may replace studies of traditional CV outcomes that take longer time and are more expensive. The method is completely non-invasive and safe, easy to use, reliable and repeatable in expert laboratories. FMD is limited mainly by technical difficulties and methodological shortcomings. Extensive training of the operator is needed resulting in a long learning curve, and image analysis may be labor-intensive. Potential environmental/physiological influences (e.g. food, caffeine, temperature, stress) need to be controlled for. Methodological standardization is needed (cuff positioning, timing of response, edge detection, software analysis, stereotactic probe-holding devices) to reduce operator-dependence, improve reproducibility and allow comparison among laboratories [161]. Normal and reference values are yet to be established.

Evolution of the methodology is sought to establish applicability in daily practice and acceptable cost/benefit ratio. Furthermore, the reclassification of subjects at intermediate risk is still open to question. Therefore, currently FMD remains mainly a valuable research tool.

Current status in clinical practice guidelines:

The ESC guidelines have not endorsed FMD for clinical use either for refinement of risk stratification [24] or for monitoring the effect of treatment [23]. FMD has a class III/B indication in the ACCF/AHA guidelines for the assessment of CV risk in asymptomatic adults [52]. (Table 6)

6.2. Endothelial function assessed by recordings at the finger

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **III/C**.

Noninvasive, finger probe-based methods exist for endothelial function assessment: digital thermal monitoring (DTM), digital volume photoplethysmography (DVP) and, the most widely used, endothelial peripheral arterial tonometry (EndoPAT). DTM (Endothelix Inc., Houston, TX, U.S.A.) is a technique that shares the same pathophysiological background with FMD using temperature as a surrogate marker of blood flow; nevertheless few studies with small sample sizes have been published to date [162,163], Similarly, DVP has been used for endothelial function assessment on top of its use for pulse wave analysis [164], but there are limited published data to date [165].

The pathophysiological background of EndoPAT is similar to that of brachial FMD, since both assess endothelial function. Nevertheless, several studies have reported low to moderate correlation between the two methods, suggesting that FMD and EndoPAT provide distinct information regarding vascular function [166–168]. These findings may be explained by the corrections implemented in the EndoPAT (see Methodology section below), whilst no such correction is incorporated in the brachial FMD method, and by the difference in type of vessels measured – small arteries and microcirculation in the case of EndoPAT versus single conduit vessel in the case of brachial FMD [166,167,169].

Methodology:

EndoPAT is based on the noninvasive measurement of pulsatile volume changes at the fingertip by peripheral arterial tonometry using a patented device (Itamar Medical, Caesarea, Israel). Essentially, the method is a modification of volume plethysmography. Tests can be carried out with the patient positioned either sitting or supine, with the sensors placed on the index finger of each arm. The endothelium-mediated changes in the finger vasculature elicited by a 5-min occlusion of the brachial artery are quantified, using a standard BP cuff inflated to a suprasystolic pressure. When the cuff is abruptly released, the surge of blood flow causes an endothelium-dependent FMD. The dilation is captured by the device as an increase in amplitude of the peripheral arterial tonometry signal. A post-occlusion to pre-occlusion ratio is calculated. Because the signal is also affected by additional, non-endothelial dependent factors, those are corrected by measurements in the contralateral arm. An additional correction is performed for baseline values. Thus, the corrected values characterize the bioavailablity of NO and therefore represent endothelial function. A score is provided in either linear or logarithmic scale.

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* EndoPAT has been incorporated in numerous population-based studies such as the Framingham, the Gutenberg Heart and Heart Strategies Concentrating on Risk Evaluation (HeartSCORE) study where it was shown to correlate with conventional risk factors of atherosclerosis and diabetes [166,167,170,171]. In coronary artery disease, it distinguished high from moderate and low risk patients [172]. In patients referred to diagnostic angiography, the calculated score was worse in patients with coronary artery disease than in patients without [173]; the score predicted both obstructive and non-obstructive ischemic heart disease [174]. Similarly to FMD, EndoPAT score is very sensitive in response to risk factors or, conversely, upon initiation of treatment. (Table 2)

2. *Prospective validation and* 3. *Incremental value:* EndoPAT was predictive of outcomes in 2 studies in heart failure patients with preserved ejection fraction followed for 5 and 20 months respectively [175,176]. With respect to primary prevention, there is, to date, one published study demonstrating the ability to predict future CV events during a 7-year follow-up period [177]. In the aforementioned study, a score (natural logarithmic-scaled reactive hyperaemia index) < 0.40 was shown to have added prognostic value after adjustment for the FRS (HR: 1.68; 95% CI: 1.02–2.78) [177]. (Table 3)

4. *Clinical utility:* The clinical utility of the method is largely unknown; no studies have so far examined whether they can change predicted risk sufficiently to change recommended therapy.

5. *Clinical outcomes:* In a similar fashion to FMD, EndoPAT can be modified with drug and lifestyle interventions [178–182]. Nevertheless, it is unclear if such modifications will eventually result in a reduction in adverse events; such randomized clinical trials are awaited.

6. *Cost-effectiveness:* There are no available data regarding cost-effectiveness.

7. *Ease of use:* The method is relatively easy to use, in an operator-independent manner.

8. *Methodological consensus:* Measurements are uniformly performed according to the manufacturer's instructions; nevertheless, guidelines do not exist yet.

9. *Reference values:* There are no reference values.

Therefore, EndoPAT does not fulfill the 9 essential criteria in order to be considered a clinical surrogate endpoint (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

Endothelial function assessment by finger recordings is noninvasive, quick and easy to perform and is both operator and interpreter-independent. Reproducibility of measurements, minimum training requirements and a strong pathophysiological basis are ascribed among its advantages. The disadvantages of the method include the moderate correlation with FMD, which raises issues regarding the specificity of the test for endothelial function, the need for a proprietary device, the cost of single-use finger probes, as well as the lack of normal/reference values and of randomized clinical trials addressing prospective validation, incremental value and clinical outcomes issues.

Endothelial function assessment with the EndoPAT method is not limited by methodological variability, as is the case with FMD, but it still lacks a solid body of clinical evidence.

Current status in clinical practice guidelines:

Endothelial function assessment by recordings at the finger is not endorsed by current guidelines (Table 6).

7. Circulating biomarkers related to vascular wall biology

Usefulness for primary and secondary CV disease prevention (Recommendation/Level of evidence): **IIb/B for high sensitivity C-reactive protein**.

A vast array of biomarkers related to different aspects of CV pathophysiology has been proposed as candidates for refinement of risk prediction. The present review focuses on biomarkers that *are related to vascular wall biology*; therefore, cardiac troponins and natriuretic peptides that are the most widely validated and used biomarkers in clinical practice remain outside its scope. C-reactive protein (CRP) is, at present, the only circulating biomarker related to vascular wall biology with a large body of published studies supporting its clinical use for risk stratification. Other novel circulating biomarkers, including oxidized low-density lipoprotein [183–185] and dysfunctional high-density lipoprotein [186–188], have a future potential for prevention but are not discussed owing to their currently limited bench-to-bedside implementation.

High-sensitivity CRP.

High-sensitivity CRP (hsCRP) is a marker of systemic inflammation that is up regulated as a consequence of vascular disease

[189]. Recent data have revealed that apart from its hepatic origin, CRP is present in both atherosclerotic plaques and injured vessel walls, which are involved in its secretion (small amounts) [190].

Fulfilment of surrogate endpoint criteria:

1. *Proof of concept:* hsCRP levels are correlated to traditional risk factors, such as systolic BP, lipids and body mass index, as well as other inflammatory indices, such as white cell count, interleukin-6 (IL-6) and fibrinogen levels.

2. *Prospective validation and* 3. *Incremental value:* A multitude of studies have examined the predictive potential of CRP above and beyond classical risk factors (Table 3). In the largest individual data meta-analysis to date (n = 160,309), the risk ratio for coronary heart disease per 1-SD higher log(e) CRP concentration (three-fold higher) was 1.37 (1.27–1.48) when adjusted for age, sex and conventional risk factors [191]. The same study group reported that the addition of CRP to traditional models for the 10-year risk prediction improved the C-index modestly, yet significantly by 0.0039; the NRI and integrated discrimination index (IDI) were 1.52% and 0.0036 respectively [192].

4. *Clinical utility:* The clinical utility of CRP, i.e. whether it can alter predicted risk sufficiently to change recommended therapy, has been tested in a statistical model derived from the aforementioned meta-analysis. It has been demonstrated that 5.2% of people >40 years old initially at intermediate CV risk would be reclassified in the highest risk category and, thus, be eligible for statin therapy [193] (Table 3).

5. *Clinical outcomes:* Regarding clinical outcomes, the Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial reported that using hsCRP as a goal for statin therapy, even in the absence of dyslipidaemia, results in favourable outcomes [194]. Despite receiving strong criticism, JUPITER reinforced the relationship of inflammatory mediation and hard CV endpoints. Results from the ongoing Cardiovascular Inflammation Reduction Trial (CIRT) and Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) will further elucidate the effect of targeted anti-inflammatory therapies on CV outcomes.

6. *Cost-effectiveness:* At present, the cost-effectiveness of routinely measuring hsCRP levels for risk stratification has not been assessed.

7. *Ease of use:* hsCRP measurement is easy and widely available.

8. *Methodological consensus:* hsCRP measurement is standardized.

9. *Reference values:* A threshold of 2 mg/L has been proposed for CV risk assessment [3]. Nevertheless, a single cut-off value may not be ideal, because inter- and intraindividual variability exists. This is attributed to genetic polymorphisms, post-translational mechanisms and presence of comorbidities that can alter baseline values [189].

Therefore, hsCRP meets some of the 9 essential criteria to classify as a clinical surrogate endpoint (Tables 4 and 5).

Advantages/disadvantages, issues remaining to be addressed and future perspectives:

The role of hsCRP for prediction of future CV events is firmly established by large trials and meta-analyses. Adoption into clinical practice is facilitated by easy, standardized measurements using widely available analytical assays. Nevertheless, due to the fact that this is an acute phase reactant, elevated hsCRP levels lack specificity for CV disease. In addition, it is unclear if their relationship is causal or an epiphenomenon; this is a pertinent question soon to be elucidated by ongoing studies of anti-inflammatory therapies.

Current status in clinical practice guidelines:

According to the ESC guidelines for CV disease prevention in clinical practice, hsCRP levels may be measured as part of refined risk assessment only in patients with an unusual or moderate risk profile (class IIb/B recommendation), but not in asymptomatic low-risk or high-risk individuals (class III/B recommendation) [24]. The 2010 ACCF/AHA guidelines advocate hsCRP measurement for selecting patients for statin therapy according to the JUPITER trial inclusion criteria (class IIa/B recommendation), for risk assessment in asymptomatic men <50 years old/women <60 years old (class IIb/B recommendation), but not in asymptomatic high-risk adults (class III/B recommendation) [52]. The most recent ACC/AHA guidelines state that hsCRP measurement may be considered if, after quantitative risk assessment, a risk-based treatment decision is uncertain (class IIb/B recommendation) [3]. Regarding dyslipidaemias, the European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines do not recommend measuring hsCRP as a secondary target of therapy for everybody; however, it may be useful in people close to the high-risk category to better stratify their total risk [195]. In the recently published ACC/AHA guidelines for the treatment of blood cholesterol, a hsCRP level >2 mg/L is an additional factor to be considered during treatment decision making for individuals without atherosclerotic disease (class IIb/B recommendation) [196] (Table 6).

8. Interplay between macro and microvascular disease

Relationships between structural changes in the micro and macrocirculation

Due to the viscoelastic properties of large arteries, the pulsatile pressure and flow that result from intermittent ventricular ejection is smoothed out, so that microvasculature steadily mediates the delivery of nutrients and oxygen to tissues [197]. The disruption of this function leads to end-organ damage. Microvascular structure is not only the site of vascular resistance; it may also substantially contribute to the wave reflections generating increased central systolic BP in the elderly [197], although the proper location of a reflection site may be elusive [198].

The structure of subcutaneous small resistance arteries, as assessed by their media/lumen ratio, correlates both with wave reflections, stiffness of large conduit arteries [199] and BP levels; in a population of more than 200 normotensive subjects and hypertensive patients, clinic systolic, diastolic, mean 24-h systolic and diastolic BP, as well as the pulse pressure/stroke volume ratio (an index of large artery compliance) correlated with media/lumen ratio [200]. Furthermore, it holds a prognostic role, as media/lumen ratio and pulse pressure (a rough index of large artery stiffness) were the two most important factors in predicting the outcome of hypertensive patients [201]. Large artery stiffness is related to cerebral microvascular disease (lacunar infarctions/large white matter hyperintensities) [202,203]. Using a non-invasive approach (scanning laser Doppler flowmetry) for the evaluation of retinal arteriolar morphology, it has been demonstrated that central pulse pressure and AIx correlate with wall/lumen ratio [204].

Such relationships indicate a coupling and crosstalk between micro- and macrovascular arterial beds [205]. Increased media/lumen ratio and rarefaction of capillaries are early manifestations of target organ damage [206], as well as major factors for an increase in mean BP, which, in turn, increases large artery stiffness through the loading of stiff components of the arterial wall. The increased large artery stiffness amplifies pulse pressure, which, subsequently, damages small arteries of the heart, brain, retina and kidney. Thus, the crosstalk between small and large arteries exaggerates target organ damage, following a vicious circle [205].

Studies have demonstrated an improvement or even an almost complete normalization of the structure of subcutaneous small resistance arteries with ACE-Is and angiotensin II receptor blockers.

In contrast, atenolol and hydrochlorothiazide were devoid of such effects [207].

In conclusion, CV events are the consequence of vascular damage at both the macro and microcirculatory level. The presence of structural alterations of small resistance arteries may be associated with the increase in large artery stiffness and possibly contribute to an increase in central pressure by increasing the magnitude of wave reflections.

9. The role of vascular biomarkers in specific subgroups (Table 7)

9.1. Hypertension

9.1.1. Prediction of developing hypertension in normotensive adults

Endothelial dysfunction, as assessed by FMD, has been shown to provide independent predictive value on the incidence of hypertension [208–210], although these findings were not always independent from baseline BP levels [210].

Of all the herein discussed vascular biomarkers, arterial stiffness most strongly associates with hypertension. Arterial stiffness is considered to have a bidirectional causal relationship with BP. On the one hand, it is proposed that elevated BP has a damaging effect on the arterial wall thus accelerating the stiffening process; for that reason high PWV is regarded as BP-related target organ damage. On the other hand, arterial stiffening is regarded as the principal cause of increased systolic BP in the elderly. The later hypothesis is now supported from 8 prospective epidemiological studies including collectively over 18,200 individuals ranging 30–80 years of age [209,211–214]. All these studies, independently from the method that was used to assess arterial stiffness (local carotid elasticity by ultrasound [211], aortic stiffness by ultrasound [212] or magnetic resonance imaging [215], baPWV [213,214,216] or cfPWV [209,217], small artery elasticity determined by pulse contour analysis [215]), showed that arterial stiffness can predict the incidence of hypertension independently from baseline BP level and age. The increase in the risk for future development of hypertension ranged from 15% [211] to 30% [209] per 1-SD increase in arterial stiffness, or 10% per 1 m/s increase in PWV [217]. So far there are no established cfPWV cut-off values in order to recommend more intense prevention strategies in individuals at high risk to develop hypertension. One study indicated the cut-off of 5.8 m/s for cfPWV [217], which clearly depends on the population's age and the applied methodology, whereas a cut-off value around 13 m/s for baPWV has been indicated by two studies [214,216]. Specifically designed trials are warranted; it should be noted that the aforementioned values refer to the predictive ability of cfPWV and baPWV for subsequent development of hypertension and not CV event prediction.

Scarce data on the ability of other vascular biomarkers to predict the incidence of hypertension exist for wave reflections [209], and cIMT [215]. Of note, one study showed that cfPWV, AIx and FMD predicted the incidence of hypertension independently of each other [209]. Very limited negative evidence exists regarding the ability of CRP to predict the development of hypertension [216].

9.1.2. CV risk stratification in hypertensive individuals

The ESH/ESC guidelines for the management of arterial hypertension encourage the screening for asymptomatic organ damage as an intermediate stage in the continuum of vascular disease [23].

So far, among all vascular biomarkers, FMD is the only one supported by a clinical study (although non-randomized) showing that endothelial function improvement with antihypertensive drug treatment is associated with a reduction in events in hypertensive postmenopausal women [158]. However, no specific data exist on the ability of FMD to reclassify CV risk in hypertensives.

Regarding arterial stiffness, the ESH/ESC guidelines for the management of arterial hypertension focus on the gold-standard index, the cfPWV, suggesting that a cut-off value above 10 m/s (when the distance is assessed as 80% of the direct distance) should be considered as evidence of subclinical organ damage [23]. Three cohorts with hypertensive individuals (total 3300 persons) provided data on the independent predictive ability of cfPWV [81,218] and baPWV [219] for all-cause mortality and CV events/mortality. Of note, the predictive ability of cfPWV in hypertension is valid throughout the whole age range [89], which is not the case for other specific pathologies, such as end-stage renal disease. However, although in one subanalysis the ability of cfPWV to predict primary coronary artery disease events above the FRS was shown [80], no study so far has addressed the specific issues of discrimination and/or reclassification in hypertension. It is advisable that the reclassification data (15%–19%) [91,92] that are derived from general population studies (approximately 30% of the study population were hypertensives with no other risk factors) or other selected populations, should be cautiously extrapolated to individuals with essential hypertension. The most recent individual-data meta-analysis on the ability of cfPWV to predict mortality and CV events showed that there is no difference between individuals with and without hypertension; the same study showed an overall 13% reclassification [88]. The treatment benefit for hypertensives after risk reclassification by cfPWV remains open to question. The selection of most appropriate BP lowering drugs with more established “de-stiffening” properties is reasonable, but data on earlier drug treatment initiation, more aggressive BP lowering or addition of other major CV risk prevention drugs (statins and/or aspirin) are not available.

Regarding the ability of wave reflections and central haemodynamics to reclassify CV risk in hypertensives, the data are heterogeneous and limited; a recent a meta-analysis included all the available data from a variety of high risk populations [131]. However, in terms of clinical utility, one cannot neglect the fact that different classes of antihypertensive drugs have variable effects on wave reflections and central BP that are accompanied with target organ damage regression [134,135]; these effects cannot be always monitored from the brachial pressure or anticipated by arterial stiffness that may remain unchanged [129,220]. Indirect evidence on the clinical value of this fact was provided by the CAFÉ study, where, despite a similar reduction in peripheral systolic BP, a calcium-channel blocker regimen was more effective in lowering central systolic BP and reduced future CV events compared with a beta-blocker regimen [129]. In the aforementioned study, excess pressure integral (XSPI), a novel central haemodynamic index, predicted outcomes independently from traditional risk scores and the FRS [221].

Whether cIMT and/or the presence of carotid plaque reclassify hypertensives in a clinically meaningful way is still debated. Recently, in the hypertensive subgroup of the USE-IMT individual data meta-analysis, common cIMT measurements did not show added value. Nevertheless, when only hypertensives at intermediate risk were examined, the addition of cIMT to existing risk scores determined a small, yet significant improvement in reclassification (NRI: 5.6%) [222]. The European Lacidipine Study on Atherosclerosis (ELSA) failed to show a predictive role of treatment-dependent cIMT changes in treated hypertensives, despite the added predictive role of baseline cIMT values and plaques [223].

The ability of ABI to reclassify CV risk in hypertension has not been addressed in specific cohorts of hypertensive individuals. However, an analysis of the National Health and Nutrition Examination Survey (NHANES) cohort which is comprised by almost 75% hypertensives, suggested that in CV disease-free individuals with ABI<0.9, better outcomes were observed in those who were at least

Table 7
The role of vascular biomarkers in patients with comorbidities.

	Hypertension	Dyslipidaemia	Diabetes mellitus	Peripheral arterial disease	Stroke	Renal disease	Coronary artery disease
Carotid ultrasonography	++ (for intermediate risk individuals)	+++	++	++	+++	++	+
Ankle-brachial index	++++	+++	+++	++++	+++	++/+++	+++
Arterial stiffness							
Carotid-femoral pulse wave velocity	++++	++	++ or +++	++	++++	++++	+
Brachial-ankle pulse wave velocity	++++	++	++	Non applicable	++	+++	+
Central haemodynamics/Wave reflections	++	+	++	++	++	++	+++
Endothelial function							
Flow mediated dilatation	+	++	+	+	+	+	++
Endothelial peripheral arterial tonometry	+	+	+	−	−	−	++
Circulating biomarkers related to vascular wall biology							
High sensitivity C- reactive protein	+	+++	+	+	+	++	−

on two out of three major CV protective drugs (i.e. antiplatelet agents, ACE-I/angiotensin receptors blockers and/or statins) [67]. The impact of ABI-driven therapeutic decisions in hypertensives warrants further investigation.

In conclusion, "primordial prevention" (i.e. avoidance of ever-having risk factors) and maintenance of low risk (maintaining optimal risk levels throughout life) are now promoted. The limited data derived from studies in hypertension and extrapolation of data from the general population show that cfPWV can reclassify CV risk substantially and predict the development of hypertension independently of baseline BP levels and other risk factors. Future studies should focus on lifestyle changes and assess whether these interventions can delay arterial stiffening and progression to hypertension. Carotid ultrasound has additive value in intermediate-risk hypertensives, especially when either an increased cIMT and/or the presence of plaque are sought for. Based on the limited number of studies, ABI may also have additive value in CV risk stratification. Implementation of vascular biomarkers is most useful in risk stratification among patients at intermediate risk. More studies assessing the additive value of vascular biomarkers to traditional risk factors and outcome studies with vascular biomarker-driven therapy are needed.

9.2. Diabetes mellitus

9.2.1. Prediction of developing diabetes mellitus in diabetes-free individuals

Microvascular dysfunction has been proposed as a potential mechanism leading to insulin resistance and type 2 diabetes mellitus, because it impairs the timely access of glucose and insulin to their target tissues. This hypothesis was further confirmed by a meta-analysis showing that dysfunction of the microcirculation, as assessed by various circulating and vascular biomarkers (which are beyond the scope of this manuscript) is an independent determinant of incident diabetes mellitus [224]. On the other hand, the hypothesis of the CV continuum in diabetes suggests that alterations/dysfunction of macrocirculation take place early in the course of glucometabolic impairment, before the clinical onset of type 2 diabetes mellitus [70]. One step further, it was recently shown that the presence of carotid plaques is a predictor of incident diabetes independently of the metabolic profile, BP and related drugs [225].

9.2.2. CV risk stratification in patients with diabetes

Relatively limited studies have addressed the ability of the herein discussed vascular biomarkers to reclassify CV risk in diabetic populations.

Data derived from a meta-analysis of 4220 individuals with diabetes showed that there was no NRI with the addition of mean common cIMT to the FRS, suggesting that cIMT should not be recommended for improving CV risk stratification in individuals with diabetes [34]. Data on carotid plaque NRI are not available.

One study so far suggests that cfPWV is a robust independent biomarker that integrates and predicts mortality in diabetes [77]. Local stiffness indices at the carotid, femoral and brachial artery may provide additional mutually independent information on incident all-cause mortality and events [72]. Due to sample size limitations, no data on the reclassification ability of arterial stiffness biomarkers are available from these two studies; however, no differences between non-diabetics and diabetics have been found, thus favoring the extrapolation of data from non-diabetic populations [72].

Although concerns about the validity of the cut-off of 0.90, as well as the association between the ABI and mortality in individuals with diabetes have been raised, new evidence show that low ABI has similar clinical value in individuals without and with diabetes [226]. However, the use of aspirin or antioxidants for the primary prevention of CV events/mortality in diabetics with an ABI <1.0 is not justified [66]. Studies on statins or other CV reduction strategies are not available.

Medial calcinosis and its consequence, a high (>1.40) ABI is frequent in long-standing diabetes [58]. This condition is associated with increased risk of mortality but only when occlusive PAD coexists. The latter should be diagnosed with further investigations in case of high ABI (e.g. toe-brachial index or Doppler waveform analysis) [59,60,227]. In a recent report, high ABI was associated with increased risk of CV mortality only in diabetic patients, where toe-brachial index was mostly decreased, underlining the high frequency of occlusive PAD concealed by the medial calcinosis during the ABI measurement.

9.3. Dyslipidaemia

9.3.1. Prediction of developing dyslipidaemia in individuals with normal lipid profile

There are no studies supporting the ability of vascular biomarkers to predict the future development of dyslipidaemia.

9.3.2. CV risk stratification in individuals with dyslipidaemia

The European guidelines for the management of dyslipidaemias suggest the use of cIMT and/or plaque presence for risk stratification, in order to better define the optimal LDL treatment target [195]. Although this is a reasonable approach, since cIMT and particularly plaque presence indicate the presence of atherosclerosis, no data exist for the ability of cIMT/plaque to reclassify CV risk in individuals with dyslipidaemia. Moreover, the potential clinical value of cIMT/plaque to stratify risk within the population of familial hypercholesterolaemia remains uncertain; however, its use may be reasonable since risk within this group is highly variable [228]. To

date, no data exist on the ability of other vascular biomarkers for CV risk reclassification in individuals with dyslipidaemia.

9.4. Peripheral artery disease

The presence of PAD stratifies patients among the highest risk groups. Compared with people of the same age, patients with PAD have a significant higher percentage of risk factors and, thus, a higher CV risk [229]. Symptomatic PAD is associated with a 2- to 8-fold increase in severe CV events and nearly a fourfold increase of mortality [230,231]. CV risk in PAD patients is even more increased in the setting of polyvascular disease. The risk for CV death, myocardial infarction, stroke and hospitalisation is approximately 12% with 1 vascular bed disease and approximately 26% with 3 vascular bed disease [232]. The importance and implications of non-coronary atherosclerosis (i.e. PAD of upper and lower extremities, carotid arteries, thoracic/abdominal aorta, mesenteric and renal arteries) has been scrutinized in a review by the ESC Working Group on Peripheral Circulation [233].

The use of vascular biomarkers may further refine risk stratification for PAD patients. FMD has shown an independent predictive ability for CV events in PAD patients. In addition, patients with PAD and relatively normal endothelial function have a very low risk of events and this holds true for long-term follow up periods [223,234].

Currently, data regarding the predictive ability of PWV and EndoPAT for CV events in PAD patients are lacking. This is also true for cIMT; numerous dedicated trials underline the relationship of increased cIMT with reintervention or disease progression at different sites, but not its predictive ability for CV events in PAD [235].

ABI has an outstanding position not only as a diagnostic but also as a prognostic tool in PAD patients without medial calcinosis. Details regarding the predictive role of ABI in PAD patients are provided in the respective section of the document above.

Concerning circulating biomarkers, CRP predicts disease progression and long-term CV mortality. Patients with intermittent claudication and elevated high sensitivity CRP levels had more than 5 times higher risk for CV mortality than those with low levels of this inflammatory biomarker (HR: 5.16; 95% CI 1.78–14.8) [236].

9.5. Stroke

Stroke seems to be an equally robust biomarker as PAD is for prediction of CV events and death. A history of cerebrovascular disease is associated with an HR of 1.42 for further events [237]. Concerning risk, this is not substantially different between so called low risk patients with a non-recent transient ischaemic attack, patients with a stroke at a young age or patients with a stroke at an older age. For stroke patients, impaired FMD proved to be an independent predictor for new onset of general vascular events (HR: 3.48; 95% CI: 1.26–9.63) [238]. In stroke patients, common cIMT predicted stroke recurrence; for each 0.1 mm increase, the probability of recurrent stroke increased by 18% (CI: 2.0%–36%) in a follow-up period of 28.9 months [239]; this is also the case for ABI [35,234,240] Baseline cfPWV values have a predictive role regarding functional outcomes of stroke patients [68,87].

9.6. Renal impairment

Renal impairment is also considered as target organ damage of atherosclerotic disease and, when adjusted to the different grades of impairment, CV risk can be very well predicted [241]. As estimated glomerular filtration rate declines, the risk for CV events increases. This is particularly true in patients with PAD, where renal dysfunction predicts long-term mortality [242]. Studies in patients with chronic kidney disease [93] and end-stage renal failure [83] have shown that cfPWV can further stratify cardiovascular risk in the presence of renal impairment. Data on ability of PWV to reclassify cardiovascular risk in these populations are missing but the most recent individual data based meta-analysis on the ability of cfPWV to predict mortality and CV events showed that there is no difference regarding the ability of cfPWV to associate with outcomes between individuals with and without impaired renal function (stage 0–5) [88].

10. Pharmacological modification of vascular biomarkers and CV risk

Different drug classes, as well as non-pharmacological approaches, can alter measured levels of vascular biomarkers. (Table 8) The pertinent question is whether this modification will translate in a subsequent reduction of morbidity and mortality. The dal-HEART program constitutes an insightful example of the use of vascular biomarkers as surrogate endpoints in clinical trials. Dalcetrapib, a cholesterol ester transfer protein (CETP) inhibitor, raised HDL levels but failed to improve endothelial function (assessed by FMD) and marginally improved carotid artery remodelling in patients with, or at high risk for CAD [243,244]. In concordance with the lack of effect on vascular biomarkers, dalcetrapib did not reduce the risk of recurrent CV events [245]. A similar failure of torcetrapib to decrease the progression of carotid and coronary atherosclerosis was accompanied by increased morbidity and mortality [246,247].

Pharmacological modulation of endothelial function:

Thiazide and thiazide-like diuretics do not have an effect on endothelial function; aldosterone antagonists have a beneficial effect in the setting of hypertension but not in type 2 diabetes mellitus [248–250]. ACE-I and angiotensin receptor blockers improve endothelial function. A differential effect on endothelial function in the macro- and microcirculation has been found for some antihypertensive drugs: calcium channel blockers improve endothelial function at the microcirculatory level, while the effect on the macrocirculation is not convincing. The opposite was seen for vasodilating beta-blockers like nebivolol: an improved endothelial function mainly at the macrocirculatory level, but not convincing at the microcirculation [251]. A meta-analysis including 25 randomized controlled trials confirmed the favorable effect of ACE-I on endothelial function measured with brachial FMD, which did not differ from angiotensin receptor blockers but was superior to the effect of calcium channel blockers and beta-blockers [252]. Non-vasodilating beta-blockers have no effect on endothelial function. In contrast, the third generation beta-blockers nebivolol and carvedilol have a beneficial effect on endothelial function through preservation of NO synthase activity by reducing asymmetrical dimethylarginine and by enhancing the bioavailability of NO because of their antioxidant properties. In addition, nebivolol activates NO synthase [253]. In a similar fashion, other drugs like phosphodiesterase type 5 inhibitors [254], statins [255–257] and thiazolidinediones, such as pioglitazone [258], improve endothelial function.

Pharmacological modulation of arterial stiffness:

The large majority of antihypertensive drugs decrease arterial stiffness [74,259–261]. However, it is not always clear whether this decrease in stiffness is limited to a ‘passive’ decrease induced by a lower BP, or whether the drug has an additional active effect on the stiffness of the vessel wall. Despite the decrease in BP, a substantial amount of studies with diuretics and nonselective beta-blockers did not show a decrease in arterial stiffness. In comparative studies, for a similar decrease in BP, ACE-I had a larger effect on arterial stiffness than calcium channel blockers [262] and the combined diuretic amiloride-hydrochlorothiazide [263],

Table 8
Pharmacological modification of vascular biomarkers.

	Carotid intima-media thickness	Arterial stiffness	Central systolic blood pressure	Wave reflections (augmentation index)	Endothelial function
Angiotensin-converting enzyme inhibitors	↓	↓	cSBP > pSBP	↓↓	+[a]
Angiotensin receptor blockers	↓	↓	cSBP > pSBP/ cSBP = pSBP	↓	+/−
Direct renin inhibitors	↓	↓	cSBP > pSBP	↓	+
Aldosterone antagonists	↓	↓/-	cSBP = pSBP	↓	+
Calcium channel blockers	↓↓ (more pronounced effect may be due to larger vasodilation)	↓	cSBP > pSBP/ cSBP = pSBP	↓↓	+[b]
Diuretics	↓	↓/-	cSBP < pSBP/ cSBP = pSBP	−	−
NO donors		↓			
Nitrates		↓/−	cSBP > pSBP	↓	
β-blockers	↓	↓ (non-vasodilating); ↓↓ (vasodilating)	cSBP < pSBP (non-vasodilating); cSBP > pSBP (vasodilating, mainly nebivolol)	↑ (non-vasodilating); ↑/−/↓ (vasodilating)	±/−[c] (non-vasodilating); +[a] (vasodilating, mainly nebivolol)
α-blockers	↓			↓	
Centrally acting drugs (moxonidine)		−	cSBP = pSBP	−	−
Phosphodiesterase-5 inhibitors		↓ (short-term effect)	trend for cSBP > pSBP	↓	+
Statins	↓/−	↓ (ezetimibe has a similar effect)	cSBP = pSBP	↓	+
Anti-tumor necrosis factor α (TNFα)		↓			+
Advanced glycation end (AGE) product breakers		↓			
Thiazolidinediones	↓	↓			+

For endothelial function: ±: weak beneficial effect; +: beneficial effect; −: neutral effect.
For arterial stiffness, all effects are long-term effects (≥1 month), unless indicated otherwise; ↓: decreases arterial stiffness; −: neutral effect.
For wave reflections (augmentation index): ↓: decrease; −: neutral effect; ↑: increase.
For central systolic blood pressure the effect on central systolic blood pressure (cSBP) beyond the effect on peripheral systolic blood pressure (pSBP) is examined; cSBP > pSBP: a more pronounced effect on cSBP compared with pSBP; cSBP = pSBP: similar effect on cSBP and pSBP; cSBP < pSBP: a smaller effect on cSBP compared with pSBP.
For cIMT: ↓: decreases IMT; −: neutral effect.
[a] More pronounced effect on macrocirculation.
[b] More pronounced effect on microcirculation.
[c] Neutral effect for atenolol, weak beneficial effect for metoprolol.

suggesting a BP independent effect of ACE-I on arterial stiffness.

In short-term trials, arterial stiffness can be improved by functional changes (vascular smooth muscle relaxation) and to some extent also by structural changes. Strong vasorelaxation, as can be seen with some nitrates, however, can lead to an increase in diameter and arterial compliance without changing arterial stiffness. In long-term trials with antihypertensive drugs, structural changes are likely to be more prominently present [262,264]. In long term trials, calcium channel blockers, beta-blockers and diuretics improved arterial stiffness also after adjustment for mean BP, heart rate, gender and other CV risk factors [262], suggesting that a sustained decrease in BP per se may improve arterial stiffness. Also, in this long term study ACE-I tended to be the most effective, suggesting a direct BP independent effect of this drug class on arterial wall remodeling. Similar destiffening effects have been reported after long-term treatment with olmesartan, suggesting that renin-angiotensin system blockade, either by ACE-Is or ARBs, exerts beneficial effects on arterial stiffness [265]. Meta-analytical approaches have attempted to identify differential effectiveness of classes of drugs but results are inconclusive due to small number of studies [266].

Anti-inflammatory drugs (corticoids, anti-tumor necrosis factor agents), lipid lowering drugs (statins, ezetimibe) and phosphodiesterase type 5 inhibitors [254,267] can decrease arterial stiffness. This potential has also been demonstrated by some NO donors, antidiabetics (thiazolidinediones) and advanced glycation end product inhibitors/breakers [74,268]. However, these drugs are currently not on the market or not advised to be used as first-line drugs due to major side effects.

Pharmacological modulation of central systolic blood pressure and wave reflection:

A more pronounced decrease in central BP beyond peripheral BP has been observed in a majority of studies with inhibitors of the renin-angiotensin system (ACE-I, angiotensin receptor blockers and direct renin inhibitors), calcium channel blockers nitrates and phosphodiesterase type 5 inhibitors. The opposite was found with beta-blockers, while diuretics appear to be rather neutral [220,254,267,269–271]. Wave reflections measured by AIx were decreased by renin-angiotensin system blockers, calcium channel blockers, nitrates, phosphodiesterase type 5 inhibitors and aldosterone antagonists, while other diuretics did not change AIx [260,261,267,272]. In contrast, a majority of studies with non-vasodilating beta-blockers showed an increase in wave reflection, while vasodilating beta-blockers did not change or decreased AIx [260,273]. Other vasodilating drugs and statins may have a beneficial effect on central systolic BP and wave reflections [268].

Pharmacological modulation of cIMT and carotid plaque:

A meta-analysis of 22 randomized clinical trials on the effect of

antihypertensive drugs on cIMT, including diuretics, ACE-I, beta-blockers, calcium channel blockers, angiotensin receptor blockers and alpha blockers, showed the superiority of calcium channel blockers over other classes of antihypertensive drugs in reducing cIMT [274]. However, it cannot be sure that the larger reduction in cIMT also represents a larger reduction in intima-media wall mass. Indeed, the reduction in cIMT was also associated with a larger carotid artery diameter. It has been shown that the larger systolic carotid diameter is accompanied with a decrease in cIMT of about 25 μm versus diastolic cIMT [275]. Since no cross-sectional IMT area has been measured, it cannot be excluded that the larger decrease in cIMT and superiority of calcium channel blockers may not represent a real decrease in intima media mass. Apart from antihypertensive drugs, statins have been shown to reduce cIMT in patients with familial hypercholesterolaemia [255] and dyslipidaemia in general [276]. Nevertheless, some studies failed to show a decrease in cIMT after a 6-month treatment with statins [257,277]. Whether a more prolonged statin treatment might lead to a reduction in cIMT is not clear.

Interventions to reduce total plaque area or volume seem to promise success, since the concept of stabilizing the vulnerable plaque may be associated with a reduction of plaque volume and/or area. However, only small and limited numbers of studies are currently available and are not sufficiently reproduced by independent groups [278]. This may change if easy applicable 3D ultrasound will be used more often in the future, based on recent technical progress in 3D imaging [279].

A summary of the effect of drugs on vascular biomarkers is presented in Table 8.

Effect of lifestyle changes:

Lifestyle modification can have a positive impact on vascular biomarkers. Weight reduction improves endothelial function [280] and lowers CRP [281], oxLDL [282], cIMT [283], wave reflections and arterial stiffness [284], especially when combined with exercise [285–287], but does not influence ABI values [288]. Endurance exercise leads to improved central haemodynamics [289] and endothelial function [290]; the effect of high-intensity resistance training on arterial stiffness is detrimental [290]. Beneficial effects have been reported following smoking cessation [162].

Effect of food supplements:

Apart from lifestyle changes, different foods and food supplements have shown a beneficial effect on endothelial function: antioxidants like vitamins C and E, flavanols and polyphenols (cocoa, tea, red wine, beer), lycopene (tomato paste), grape fruit extracts and omega-3 fatty acids [251,291–294]. Some food supplements like cocoa result in decreased levels of aortic stiffness, wave reflections and central systolic BP [295]. Caffeine increases central BPs to a greater extent than peripheral BPs, as well as arterial stiffness [296].

11. Conclusion

Biomarkers should not be routinely measured during the CV risk evaluation of all patients [3,24]; this role is reserved for risk scores [4,297]. However, the addition of a vascular biomarker tends to add only modestly, yet significantly beyond classical risk factors and may be particularly useful in those patients classified as having intermediate CV risk and in whom there is a therapeutic dilemma [240]. The higher values of clinical NRIs, as opposed to overall NRIs, in studies and meta-analyses highlight the utility of biomarkers in those patients. Risk refinement, i.e. reclassification in a higher/lower risk stratum and relevant therapeutic decisions can be made by measuring one or more biomarkers. In those cases that measurement of biomarker is deemed useful, biomarkers related to the vasculature are an attractive choice since they integrate, predict risk and detect subclinical disease from different vascular beds.

The increasing number of vascular biomarkers provides complementary information that facilitates the assessment of structure, function and metabolism of arteries; such information can serve as a proxy for CV disease upon fulfillment of criteria (Tables 1 and 4). On the basis of these criteria, vascular biomarkers can be classified in three groups:

1. Those that fulfill most of the 9 essential criteria and, therefore, are close to being considered a clinical surrogate endpoint (carotid ultrasonography, ABI, cfPWV).
2. Those that fulfill some, but not all of the 9 essential criteria (baPWV, central haemodynamics/wave reflections, hsCRP) and
3. Those that do not at present fulfill the 9 essential criteria (FMD, EndoPAT) (Table 5).

Nevertheless, at this point, it is unclear if a specific vascular biomarker is overly superior. This would require a study in which all vascular biomarkers would have been measured; indirect evidence can be deducted from large studies and meta-analyses [35,227,234,298]. (Table 3) In selected cases, the combined assessment of more than one biomarker may be required [149,299]. Instead of the “one size fits all” approach, it is evident that vascular biomarkers have different strengths (Table 2**)** and choice may be dictated by the clinical setting and present comorbidities (Table 7). The ideal vascular biomarker may be different for each patient and should be further explored. Importantly, the promise of vascular biomarker-driven therapeutic decisions should be validated through randomized clinical trials data.

Conflict of interest

Dr. De Carlo reports personal fees from Abbott Vascular and Medtronic, outside the submitted work.

Dr. Mikhailidis reports to have given talks and attended conferences sponsored by MSD, AstraZeneca and Libytec.

Dr. Van Bortel reports grants from Merck, GSK, Janssen, Actogenix, Menarini and Yakult, non-financial support from Daiichi Sankyo and Servier and personal fees from Janssen and Elsevier, outside the submitted work.

Dr. Weber reports grants from i.e.m. Stolberg Germany, outside the submitted work.

Dr Boutouyrie reports grants from Atcor Medical, Alam Medical and Esaote during the conduct of the study.

Dr O'Rourke is the founding director of AtCor Medical Pty Ltd and Aortic Wrap Pty Ltd. He has received personal fees as a consultant for Merck and Novartis outside the submitted work, has an issued patent for a method of treating a stiffened blood vessel (Aortic Wrap Pty Ltd), a pending patent for a stent wrap (Aortic Wrap Pty Ltd) and a 6% holding in AtCor Medical Pty Ltd.

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