Cardiac-coronary interactions in humans: Mechanistic insights from wave intensity analysis

Rolandi, M.C.

Publication date
2014

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 2

Wave speed in human coronary arteries is not influenced by microvascular vasodilation: Implications for wave intensity analysis

M. Cristina Rolandi, Kalpa De Silva, Matthew Lumley, Timothy P.E. Lockie, Brian Clapp, Jos A.E. Spaan, Divaka Perera*, Maria Siebes*

* equal contribution

Published in Basic Research in Cardiology, 2014
ABSTRACT

Wave intensity analysis and wave separation are powerful tools for interrogating coronary, myocardial and microvascular physiology. Wave speed is integral to these calculations and is usually estimated by the single-point technique (SPc), a feasible but as yet unvalidated approach in coronary vessels. We aimed to directly measure wave speed in human coronary arteries and assess the impact of adenosine and nitrate administration.

In 14 patients, the transit time $\Delta t$ between two pressure signals was measured in angiographically normal coronary arteries using a micro-catheter equipped with two high-fidelity pressure sensors located $\Delta s = 5$ cm apart. Simultaneously, intracoronary pressure and flow velocity were measured with a dual-sensor wire to derive SPc. Actual wave speed was calculated as $D N_c = \Delta s/\Delta t$. Hemodynamic signals were recorded at baseline and during adenosine-induced hyperemia, before and after nitroglycerin administration. The energy of separated wave intensity components was assessed using SPc and DNc.

At baseline, DNc equaled SPc ($15.9 \pm 1.8$ vs. $16.6 \pm 1.5$ m/s). Adenosine-induced hyperemia lowered SPc by 40% ($p<0.005$), while DNc remained unchanged, leading to marked differences in respective separated wave energies. Nitroglycerin did not affect DNc, whereas SPc transiently fell to $12.0 \pm 1.2$ m/s ($p<0.02$).

Human coronary wave speed is reliably estimated by SPc under resting conditions but not during adenosine-induced vasodilation. Since coronary wave speed is unaffected by microvascular-induced dilation, the SPc estimate at rest can serve as surrogate for separating wave intensity signals obtained during hyperemia, thus greatly extending the scope of WIA to study coronary physiology in humans.
INTRODUCTION

Wave intensity analysis (WIA) has been developed as a time-domain method to investigate cardiovascular dynamics and derives from the local summation of individual upstream and downstream traveling wavefronts [1]. This has provided a valuable tool for studying the interaction between cardiac mechanics, dynamics of the coronary circulation and aortic hemodynamics [2-6].

Wave speed is a fundamental parameter for separating net intensity waveforms into forward and backward traveling components. Several methods exist to estimate pulse wave speed in arteries [7]. The conventional approach involves assessing the transit time between pressure waveforms obtained a known distance apart, known as the ‘time delay’ or ‘foot-to-foot’ method. However, application of this technique in the coronary circulation is difficult and to our knowledge, has never been performed in humans before. Other techniques estimate local wave speed from simultaneously acquired pressure (P) and flow velocity (U) at the same location. The PU-loop method requires a unidirectional wave period [8]. In systemic arteries, this occurs at the beginning of systole when reflected waves are absent, whereas in coronary arteries, forward and backward waves are simultaneously generated during this phase of the cardiac cycle. The sum-of-squares or single-point technique [9] does not rely on a unidirectional wave period and in the aorta, local wave speed derived by this technique (SPc) correlated well with the foot-to-foot method (pullback technique). It was proposed for use in normal human coronary vessels [9] but coronary application has not been directly validated before.

Kolyva et al. challenged the applicability of the single-point technique in coronary arteries by demonstrating that SPc did not follow the well-established relationship between wave speed and arterial stiffness [10]. Coronary SPc downstream of a stenosis paradoxically decreased when local distending pressure increased after revascularization of the proximal obstruction. Likewise, lowering microcirculatory resistance by adenosine administration markedly reduced SPc, although the wall properties of the epicardial conductance vessels would not be expected to be altered. Moreover, vasodilation of the microcirculation by dipyridamole did not alter directly measured coronary wave speed in animals [11].

The main goal of this study was therefore to assess wave speed in human coronary vessels by direct measurement at rest and during adenosine-induced maximal hyperemia, both before and after administration of nitrate. We hypothesized that in agreement with findings in animals and contrary to earlier findings for SPc, true coronary wave speed in humans is not affected by resistance vessel dilatation.

METHODS

Study population

Patients with stable anginal symptoms scheduled for percutaneous coronary intervention were recruited if at least one angiographically normal (<30% diameter stenosis) coronary vessel was present. Exclusion criteria were age under 18
years, recent myocardial infarction (<6 weeks), valvular pathology, non-ischemic cardiomyopathy, or the presence of decompensated heart failure. All participants gave written informed consent in accordance with the protocol approved by the St. Thomas’ Hospital research ethics committee (Ref: 10/H0808/58).

Cardiac catheterization and intracoronary instrumentation
After heparin administration (70 IU/kg), coronary angiography was performed using a standard Judkins technique. An 8F guiding catheter was inserted via the femoral artery engaging the coronary ostium. A 5F “mother and child” catheter (Heartrail®, Terumo, Japan) was inserted via the guiding catheter and positioned in the mid-segment of an angiographically normal coronary vessel. A 2.5F microcatheter equipped with two high-fidelity pressure sensors 5 cm apart (Mikro-Tip®, Millar Instruments, Houston, TX) was advanced to the tip of the Heartrail catheter, to obtain two simultaneous pressure waveforms (P1 and P2). These pressure signals were digitally recorded at a sampling frequency of 5 kHz (MP150, Biopac Systems, Goleta, CA). Additionally, intracoronary pressure (Pd) and flow velocity (U) were measured via a 0.014-in dual-sensor guidewire (ComboWire® XT, Volcano Corp., San Diego, CA) positioned with its pressure sensor adjacent to the distal pressure sensor of the micro-catheter. Pd and U were processed with the associated instrument console and digitized at a sampling frequency of 200 Hz. The 5F catheter was pulled back to minimize distortions in aortic pressure (Pa) via the guiding catheter. An electronic bookmark was applied on both data acquisition systems for later synchronization.

Study protocol
Hemodynamic data were obtained at rest and during adenosine-induced hyperemia (40 ± 6 μg, range 12-84 μg). These measurements were repeated after intracoronary administration of 0.1 mg nitroglycerin (NTG). In order to demonstrate sensitivity of our wave speed measurements to changes in transmural pressure, six patients were asked to perform a Valsalva maneuver (VM) at rest, which provides a convenient way to lower transmural pressure in humans [4,12] by producing a strong increase in thoracic pressure during the strain. Finally, the PU-wire was withdrawn into the ascending aorta to compare coronary with aortic wave speed.

Data Analysis
The diameter profile of the study vessel was obtained by quantitative coronary angiography (QAncio XA 7.2, Medis Medical Imaging Systems, Leiden, Netherlands). From the continuous recordings, about 10 heart cycles were selected at resting condition, 2-5 cycles at maximal flow velocity, and 2-3 at maximum strain of the VM. Local wave speed was calculated per beat and averaged over the selected beats for each condition.

Directly measured wave speed (DNc) was calculated as $$\text{DNc} = \frac{\Delta s}{\Delta t}$$, where $\Delta s$ is the 5cm distance between the two pressure sensors on the micro-catheter and $\Delta t$ is the transit time between these sites, assessed at the dicrotic notch on the two pressure waveforms, P1 and P2 (Fig. 1). The time of the dicrotic notch was determined from the local maximum of the second derivative of the respective pressure signal (AcqKnowledge 4.1, Biopac Systems, Goleta, CA).
Using a custom-made program (Delphi 2010, Embarcadero, CA), coronary $S_{PC}$ was derived from the combined $P_d$ and $U$ measurements according to the sum-of-squares method [9] as

$$S_{PC} = \frac{1}{\rho} \sqrt{\sum \frac{dP_{d}^2}{\sum dU^2}}$$

(1)

where $\rho$ is the blood density ($1060 \text{ kg} \cdot \text{m}^{-3}$). The raw signals were smoothed by a Savitzky-Golay filter [13-14] and time derivatives were obtained after correcting for the time delay between $P_d$ and $U$.

Net wave intensity ($dI$) is defined as the product of incremental changes in local pressure ($dP$) and flow velocity ($dU$) [15] and it reflects the effect of cardiac contraction and relaxation on coronary hemodynamics. Coincident forward aortic-generated ($dI_+$) and backward microcirculatory-generated ($dI_-$) travelling waves are superimposed to form the net wave intensity at the measuring location. The separate forward and backward contributions were obtained as

$$dt = \frac{1}{4\rho c} \left( \frac{dP}{dt} \pm \rho c \frac{dU}{dt} \right)^2$$

(2)

where $\rho$ is the blood density ($1060 \text{ kg} \cdot \text{m}^{-3}$) and $c$ is the wave speed (in m/s) [1].

Four different types of wave exist associated with the differential pressure and flow velocity changes during the cardiac cycle. A backward compression wave (BCW) coming from the microcirculation appears at isovolumetric contraction. The opening of the aortic valve causes the arrival of the forward compression wave (FCW) from the aorta. The relaxation of the heart generates first a forward expansion wave (FEW) coming from the aorta followed by the backward expansion wave (BEW) generated in the microcirculation [16].

To assess the effect of wave speed on the separated wave intensity during maximal hyperemia, $dI_+$ was calculated using hyperemic $DN_c$ and hyperemic as well as baseline $S_{PC}$. The respective wave energies (in $J \cdot m^{-2} \cdot s^{-2}$) were quantified by integrating the area under each of the dominant separated waves.

Figure 1: Example of two pressure signals ($P_1$ and $P_2$) recorded 5 cm apart in a coronary artery and the corresponding second time derivatives. The time delay between the peaks of the second derivatives at the dicrotic notch was used to determine actual coronary wave speed.
Statistical analysis
All values are expressed as mean ± SEM. Comparison between different conditions was performed using ANOVA with repeated measures followed by contrast analysis (SPSS v. 19.0, IBM, Armonk, NY). Agreement between DNc and SPc was assessed using Bland-Altman analysis. Wave speeds at the same condition were compared with a paired Student’s t-test. Relationships between continuous variables were investigated with linear regression. A value of p<0.05 was considered statistically significant.

RESULTS
Simultaneous hemodynamic measurements were successfully obtained in 14 patients aged 44–83 years (Table 1). In one patient, adenosine-induced hyperemia was not obtained after NTG administration. A noisy flow velocity signal prevented the derivation of SPc at baseline before NTG and in the aorta in one patient. The study vessels were minimally diseased with 12 ± 7% diameter reduction and an average diameter at the distal location of 3.0 ± 1.4 mm.

Hemodynamic variables during the protocol are summarized in Table 2. Flow velocity more than doubled after adenosine injection (p<0.001). This increase in flow velocity was not related to the adenosine dose that on average was 40 ± 6 µg (27 ± 3 µg for right and 62 ± 7 µg for left coronary arteries). NTG caused a transient flow elevation (p<0.001) about 15 sec after administration that subsided within 40–60 sec. Aortic and coronary pressures decreased by <10% (p<0.05) following NTG or adenosine injection. The pressure drop between Pa and Pd at maximum adenosine-induced hyperemia was less than 5 mmHg. The resulting stenosis resistance at hyperemia was 0.15 ± 0.05 mmHg/cm/s, which is in the order of 5% of the total hyperemic coronary resistance.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>68 ± 10</td>
</tr>
<tr>
<td>Male sex</td>
<td>9 (64)</td>
</tr>
<tr>
<td>Diameter reduction (%)</td>
<td>12 ± 7</td>
</tr>
<tr>
<td>Study vessel (LAD/LCX/RCA)</td>
<td>2/3/9</td>
</tr>
<tr>
<td>Coronary risk factors</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (71)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>10 (71)</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>3 (21)</td>
</tr>
<tr>
<td>LV function (good/poor/unknown)</td>
<td>7/1/6</td>
</tr>
<tr>
<td>Smoking History</td>
<td>6 (42)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>10 (71)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>14 (100)</td>
</tr>
<tr>
<td>b-blockers</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>5 (36)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD or n (%). LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery.
Table 2. Hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hyperemia</th>
<th>Baseline</th>
<th>NTG at peak U</th>
<th>NTG 1 min</th>
<th>NTG hyperemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>64 ± 3</td>
<td>65 ± 4</td>
<td>66 ± 4</td>
<td>64 ± 4</td>
<td>67 ± 4</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>Pa (mmHg)</td>
<td>88 ± 5</td>
<td>83 ± 5*</td>
<td>90 ± 5</td>
<td>86 ± 6</td>
<td>85 ± 4*</td>
<td>79 ± 4*</td>
</tr>
<tr>
<td>P1 (mmHg)</td>
<td>89 ± 5</td>
<td>81 ± 5*</td>
<td>89 ± 4</td>
<td>84 ± 6*</td>
<td>84 ± 5*</td>
<td>77 ± 5*</td>
</tr>
<tr>
<td>P2 (mmHg)</td>
<td>87 ± 4</td>
<td>78 ± 5*</td>
<td>87 ± 5</td>
<td>81 ± 6*</td>
<td>83 ± 4*</td>
<td>75 ± 5*</td>
</tr>
<tr>
<td>Pd (mmHg)</td>
<td>87 ± 5</td>
<td>78 ± 5*</td>
<td>88 ± 5</td>
<td>81 ± 6*</td>
<td>82 ± 4*</td>
<td>76 ± 4*</td>
</tr>
<tr>
<td>U (cm/s)</td>
<td>15.2 ± 1.0</td>
<td>34.7 ± 3.6*</td>
<td>16.6 ± 1.0</td>
<td>31.6 ± 3.8*</td>
<td>16.8 ± 1.1*</td>
<td>32.7 ± 3.3*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. NTG, nitroglycerin; U, blood flow velocity. *p<0.05 compared to the previous baseline; †p<0.05 compared to NTG at peak U.

Figure 2: Response to intracoronary adenosine-induced microvascular vasodilation.
Tracings show continuous recordings of ECG, aortic pressure (Pa), proximal (P1) and distal (P2) micro-catheter pressure, and distal pressure (Pd) and velocity (U) measured by dual-sensor guide wire. Per-beat values of wave speed (c) assessed by transit time (DNC) and by single-point technique (SPc) are shown in the bottom panel. The increase in mean flow velocity is mirrored by a decrease in SPc, while DNC remains unchanged.
Coronary wave speed

Figure 2 shows representative recordings and the beat-by-beat wave speed derived with the two methods. SPc was similar to DNc at rest. An almost three-fold flow velocity increase during adenosine-induced hyperemia did not alter DNc, but markedly reduced SPc.

Variations in wave speed induced by adenosine and NTG are summarized in Fig. 3. At baseline, the two methods yielded statistically similar results, with DNc = 15.9 ± 1.8 m/s and SPc = 16.6 ± 1.5 m/s. However, SPc dropped by almost 40% to 10.5 ± 0.9 m/s (p<0.001) during adenosine-induced hyperemia while DNc remained unchanged. Notably, DNc at maximum hyperemia did not differ from SPc at rest. All values returned to baseline. Subsequent NTG administration did not affect DNc, but SPc was reduced by 31% to 12.0 ± 1.2 m/s at peak flow velocity (p<0.001) and returned within 60 sec to a steady value that was 14% lower than the previous baseline (p<0.05). Administration of adenosine in the presence of NTG again markedly diminished SPc to 10.9 ± 1.5 m/s (p<0.005), while DNc did not change. Figure 4 illustrates the transient effect of NTG on flow velocity and coronary wave speed assessed by SPc, while DNc remains constant.

Bland-Altman analysis confirmed the equivalence of SPc and DNc at baseline with a mean difference of 0.76 ± 1.2 m/s, whereas SPc was 6.1 ± 1.9 m/s lower during hyperemia (Fig. 5). This underestimation with SPc was related to average wave speed (r=0.72, p<0.005). Figure 6 shows that coronary DNc during basal conditions was strongly related to aortic SPc (p<0.02, r=0.66). Coronary DNc was approximately 32% higher than aortic SPc, with a mean difference of 3.1 ± 4.9 m/s (p<0.05).

Effect of wave speed on the energy of separated waves during hyperemia

While wave energies assessed with SPc or DNc were equivalent at baseline, the lower wave speed estimated with SPc at maximal hyperemia led to changes in the calculated energy of the associated dominant waves (Fig. 7). Compared to the values obtained using DNc, the compression waves BCW and FCW derived with hyperemic SPc increased by 30% and 17%, respectively (p<0.02). The FEW during early relaxation was not significantly affected. In particular the BEW, considered to be an important driver of diastolic coronary flow, was estimated 7% lower than that obtained with DNc (p<0.05). When using the value of SPc obtained at baseline for wave separation at maximal hyperemia, we found no differences compared to the wave energies obtained with hyperemic DNc.

Coronary wave speed during changes in transmural pressure

Figure 8A illustrates coronary pressure and per-beat DNc throughout two consecutive VMs. The expected trend in thoracic pressure is shown schematically. Changes in DNc reflect the decline of transmural pressure during the strain and parallel its return to baseline values during the release phase. As depicted in Fig. 8B, SPc decreased from 15.7 ± 1.2 at baseline to 7.0 ± 0.7 m/s during maximal VM strain and DNc from 15.1 ± 2.3 to 7.5 ± 1.3 m/s (p<0.01). No differences were observed between SPc and DNc at baseline or during VM strain.
**Figure 3:** Effect of resistance and conductance vessel vasodilation on coronary wave speed assessed by transit time (DNc, solid bars) and by the single-point technique (SPc, hatched bars). At baseline, SPc and DNc have comparable values. At peak elevated flow induced by adenosine or by nitroglycerin (NTG), SPc is markedly reduced, while DNc is unaffected. After the transient effect of NTG had waned within one minute, SPc returns close to the baseline value. Subsequent adenosine administration again lowers SPc to a similar level as obtained at peak flow during NTG-induced microvascular dilation or prior adenosine-induced hyperemia. *p<0.005 compared to the previous baseline; †p<0.05 compared to DNc at the same condition; ‡p<0.05 compared to baseline before NTG.

**Figure 4:** Effect of NTG on coronary wave speed. Transit-time wave speed (DNc) remains essentially constant, while single-point wave speed (SPc) decreases during the transient increase in flow velocity.
Figure 5: Bland-Altman plots comparing SPc and DNc (A) at baseline and (B) during hyperemia. At baseline no differences are observed between SPc and DNc. Differences during hyperemia are related to average wave speed. Horizontal lines indicate mean and 95% confidence interval (± 1.96 SD).

Figure 6: Correlation between transit-time coronary wave speed (DNc) and aortic wave speed. Coronary measured wave speed (DNc) and aortic wave speed are related (p<0.02, r=0.66). Dashed line indicates identity.
Figure 7: Separated wave energies at peak hyperemia assessed with both wave speed methods. Wave energy changes when hyperemic SPc is used in the derivation, but is equivalent between hyperemic DNC and baseline SPc. BCW, BEW, backward compression and expansion wave; FCW, FEW, forward compression and expansion wave. *p<0.05 compared to wave energy derived with DNC.

Figure 8: Effect of changes in transmural pressure on coronary wave speed. (A) Example of variation in coronary pressure and transit time wave speed (DNc) during two consecutive Valsalva maneuvers. DNc varies in parallel with transmural pressure. Sketched changes in thoracic pressure (Pthorax, bold line) illustrate the reduction in transmural pressure during the strain phase. (B) Comparison of single-point (SPc) and transit time (DNc) wave speed at baseline and during maximal strain. *p<0.001 compared to baseline.
DISCUSSION

We assessed pulse wave velocity in the human coronary circulation by the transit time of the pressure signal in angiographically normal epicardial vessels. Directly measured coronary wave speed (DNc) was independent of microvascular or large artery vasodilation, but was sensitive to changes in vessel distensibility as demonstrated in response to the reduction in transmural pressure induced by the VM. Results obtained with the single-point technique (SPc) were equivalent at baseline, but underestimated actual wave speed during elevated flow following administration of NTG or adenosine. Notably, SPc at baseline was not different from DNc at hyperemia, which offers promising potential to extend the use of SPc for coronary wave intensity analysis to hyperemic conditions.

Nitroglycerin-induced vasodilation and coronary wave speed

Pulse wave velocity (PWV) is related to biophysical properties of the vascular wall and blood according to the Moens-Korteweg equation,

$$PWV = \sqrt{\frac{h \cdot E_{\text{inc}}}{2rp}}$$

where $E_{\text{inc}}$ is the incremental elastic modulus of the vessel wall, $h$ the wall thickness, $r$ the vessel radius and $\rho$ the density of blood. The speed at which pulse waves travel in the arterial system is a measure of arterial stiffness, which in turn is a function of transmural pressure [11]. Physiological and pharmacologic alterations can influence PWV via one of these factors. Arterial distensibility is also influenced by changes in vascular tone [17]. Elevated flow reduces tone via shear stress-mediated endothelial production of nitric oxide and may therefore influence PWV. Naka et al. observed that PWV in the upper limb of normal subjects was reduced by 14% following distal hyperemia, compared to a 5% reduction in patients with endothelial dysfunction [18]. In contrast, endothelium-independent smooth muscle relaxation by sublingual nitroglycerin reduced PWV by about 10% in both the normal and diseased group.

The situation in coronary vessels is more complicated due to autoregulation, which maintains baseline flow at a level commensurate with metabolic demands. NTG primarily dilates coronary arteries >200 μm and produces a marked but transient increase in coronary flow [19]. Jones et al. [20] observed a significant dilation of both small coronary arteries and arterioles <100 μm early after NTG administration, resulting in a substantial decrease in microvascular resistance. This dilation was sustained over several minutes for arteries, but was short-lived for arterioles as the result of autoregulatory escape mechanisms primarily operative in arterioles. On the other hand, adenosine exerts a potent vasodilator effect primarily on microvessels <150 μm. Our hemodynamic findings agree with the effect of nitrates on coronary microvessels, showing a transient increase in flow velocity that subsided within 1 min.

At variance with the unchanged DNc after NTG, we observed a transient decrease in SPc that mirrored the temporary increase in flow velocity. SPc recovered within 1 min after the NTG bolus, when coronary flow had returned to pre-NTG values, although it remained slightly depressed. Davies et al. also described a 43%
reduction in SPc with a nadir several seconds after intracoronary infusion of 1 mg isosorbide dinitrate [9]. They interpreted this to reflect an increased vascular distensibility as observed in systemic arteries over longer periods of time [17-18]. The timing of their measurements shortly after nitrate infusion likely coincided with the period of elevated flow, providing a possible alternate explanation for the observed decrease in SPc, as discussed below. Considering the prevalence of clinical risk factors in our subjects, the limited effect of NTG on DNc may be associated with a general decrease in coronary elasticity [21]. Moreover, the concomitant changes in elasticity and radius may have compensated each other so the effect of the dose of NTG given in this study remained unnoticed.

Discrepancy between wave speed assessed by the transit time and single-point method
SPc assessed in the present study was similar in magnitude to that reported previously for basal and hyperemic conditions [9-10]. Good agreement between DNc and SPc was found at baseline and both wave speed methods displayed a similar dependency on transmural pressure variations during the VM. However, during elevated flow induced by NTG or adenosine, DNc remained unchanged, while SPc decreased. There is no biophysical mechanism that could account for a decrease in wave speed by 30% in epicardial vessels after microvascular dilation with adenosine. An explanation for this apparent decrease in local wave speed assessed by SPc at elevated flow may be found in the windkesselness of the coronary arteries [10]. The formula for SPc was derived on theoretical grounds, based on minimization net wave energies over the cardiac cycle [9]. The resulting expression in terms of $\Sigma dP^2$ and $\Sigma dU^2$ (Eq. 1) is dominated by systolic-diastolic variations of the pressure and velocity waveforms that are more related to a lumped-parameter intramyocardial pump model rather than wave transmission characteristics [10]. Hence, the ratio of summed squares of pressure and velocity differentials is altered by changes in the pulsatile waveforms that are unrelated to local wall distensibility. Larger velocity variations seen during elevated flow tend to increase the denominator, while a sustained reduction in pulse pressure, observed in some patients after NTG administration, reduces the numerator. Both serve to lower SPc without representing an actual change in local wave speed.

Effect of transmural pressure alterations on wave speed
Vessel distensibility was increased in a subset of patients who performed a VM, which readily decreases aortic and coronary vessel transmural pressure [4,12]. The concurrent and similar reduction in both DNc and SPc confirmed the ability of both methods to detect changes in vessel distensibility.

Comparison of coronary DNc with aortic SPc
Aortic wave speeds found in our study are consistent with those reported by others [9,12]. The strong correlation of baseline coronary DNc with SPc in the aorta attests to the intra-subject consistency of wave speed in different vascular beds.
Wave speed effect on wave intensity analysis

Wave speed estimated by SPc from simultaneously obtained pressure and velocity signals during basal conditions appears to be a robust approximation for use in the separation of coronary wave intensity. However, SPc obtained from hyperemic pressure and velocity waveforms is prone to introduce erroneous self-cancelling waves in the separated wave intensity profiles, leading to inaccuracies in the derived wave intensities and wave energies [16]. The present results showed that the diastolic, microcirculatory originating (backward) expansion wave (BEW) is likely to be underestimated and both the microcirculatory-originating (backward) and the aorta-originating (forward) compression waves were overestimated. The deviations in wave energy due to underestimation of the actual hyperemic wave speed by SPc approached those seen as a result of pathological conditions [2,6]. Importantly, however, the hyperemic wave energies derived using SPc obtained at baseline did not differ from those derived using DNc at hyperemia. This suggests that the sum-of-squares technique can be used for coronary wave intensity analysis at elevated flow rates induced by vasodilation, as long as SPc obtained at baseline is used for wave separation.

Study Limitations

Intracoronary guide wires and catheters can potentially affect adrenergic smooth muscle control, which may alter local wave transmission characteristics. However, we did not observe any changes in coronary hemodynamics or diameter suggestive of vasospasm during the protocol and no patients encountered ischemic symptoms or ECG changes. Furthermore, the methodology adopted in this study represents the most accurate way of comparing directly (DNc) and indirectly (SPc) assessed wave speed in the human coronary artery with currently available technology. Intravenous adenosine is frequently advocated to induce hyperemia. However, since the measurement of DNc and SPc required only a few beats, the stable duration of the signals during intracoronary adenosine-induced hyperemia was sufficient for drawing our conclusions. Moreover, a disadvantage of intravenous adenosine for our study would have been the variable effect on systemic pressure, which in general is reduced by systemic vasodilation and can elicit pressure control mechanisms that may interfere with wave generation and propagation [22-23]. The adenosine-induced increase in flow velocity observed in our study group was 2.3 fold which is small for a normal coronary artery but still above the clinical threshold of 2. The administered adenosine dose averaged 40 ± 6 µg, which has been shown previously to elicit an equivalent albeit more short-lived flow response as intravenous adenosine [24-25]. We also found no relation between coronary flow velocity reserve (CFVR) and adenosine dose and hence, there must have been other factors responsible for the relatively modest increase in flow velocity. Our patients were scheduled for PCI in a diseased vessel other than the study vessel. The presence of additional risk factors and comorbidities likely explains the observed CFVR values. Furthermore, recent studies have demonstrated that adenosine often fails to induce maximal coronary dilation [26], especially in the presence of endothelium-mediated microvascular vasoconstrictors that are exacerbated by stenting [27].

The mix of data from left and right coronary arteries seems justified for this study
on the effect of microvascular dilation which was present in all cases. Also the quality of the flow velocity signals was sufficiently high, except for 1 patient at baseline that was excluded, to arrive at strong statistically significant differences between SPc at baseline and hyperemia. It should be noted that SPc cannot be obtained by devices that do not acquire high-frequency dynamic flow velocity waveforms during a cardiac cycle, such as those used for the thermodilution method which measure temperature changes over several beats.

The time delay Δt is determined by identification of a fiducial point, typically the systolic ‘foot’, on the pressure waveform. However, confluence of waves from both proximal and distal ends tends to disturb detection of this time point [28]. The dicrotic notch was shown to represent a less variable time reference in the carotid artery [29] and may well be a suitable time-point on the coronary pressure waveform. Despite consistency of our results with changes in arterial distensibility, some bias may have been introduced due to possible non-unidirectional wave travel. However, coronary reflections at frequencies relevant for wave travel were found to be insignificant [11].

We could not assess wave speed in a vessel segment downstream of a focal stenosis. As shown previously, SPc suffers from shortcomings in conditions where the pressure or velocity pulsations are increased without changes in local elastic wall properties [10]. Wave intensity analysis in diseased vessels should therefore be restricted to net wave intensity.

At peak hyperemia, resistance proximal to the site of measurement was less than 5% of microvascular resistance. While this may have contributed to the inter-patient variation in the values of DNc and SPc of about 10%, it does not affect our conclusions on the effect of microvascular dilation on SPc and the absence of such effect on DNc, since these were measured in the same patients.

**Clinical Implications**

Local wave speed in conduit arteries cannot be directly predictive of microvascular disease since it is fully determined by biophysical properties and dimensions of the vessel wall at the location where it is measured. Insofar as there is a correlation between atherosclerotic involvement in the conduit vessels and microvascular dysfunction [30], wave speed in epicardial vessels may have some predictive value for microvascular disease. However, this would require further exploration in a larger group of patients with a reference measurement for vessel wall disease. Our current findings are clinically relevant since wave speed is needed for separating forward and backward running waves and it was recently shown that coronary WIA may predict functional recovery after myocardial infarction [6]. Since we have established that SPc at baseline can be used as a surrogate for wave speed in hyperemic conditions it is now possible to fully apply wave intensity analysis (WIA) during maximal hyperemia in epicardial vessels without a focal lesion. This opens a wide range of opportunities to study the physiology of the coronary circulation and cardiac-coronary interaction in the presence of microvascular diseases.

Theoretically, self-canceling forward and backward waves may be introduced by a faulty wave speed used to separate the net intensity profile [16], which could be detrimental to the theory underlying the instantaneous wave-free ratio (iFR).
Since iFR is assessed at baseline, our results support the applicability of SPc to separate forward and backward coronary waves to identify the “wave-free” period in diastole. Theoretically, this can be extended to hyperemia using baseline SPc as a surrogate for hyperemic wave speed. However, a drawback of SPc lies in its failure to correctly represent wave speed when assessed in a vessel segment downstream of a stenosis, where a paradoxical inconsistency of SPc with pressure-dependent wall properties was demonstrated [10]. While this does not appear to introduce additional waves during periods in which no waves are present [16], a potential influence on the determination of the diastolic wave-free period for iFR should be further investigated.

**CONCLUSION**

This work is the first study in humans to determine coronary wave speed by the transit time of the pressure signal and with the single-point technique. The latter approach has the important practical advantage that coronary SPc can be assessed using a pressure and velocity sensor-equipped guidewire that also serves to determine the functional significance of a stenosis. Our results confirmed that SPc reliably appraises coronary wave speed in angiographically normal vessels under resting conditions. However, this technique markedly underestimates actual wave speed at elevated flow during transient or prolonged distal vasodilation, while directly measured wave speed was not affected. We conclude that in angiographically normal vessels, SPc assessed during basal conditions may be used to separate wave intensity profiles derived from hyperemic pressure and flow waveforms, thereby extending the utility of wave intensity analysis for coronary physiological assessment to hyperemic conditions.
REFERENCES
