Patient-specific blood pressure correction technique for arterial stiffness

Citation for published version (APA):

Spronck, B., Delhaas, T., De Lepper, A. G. W., Giroux, J., Goldwasser, F., Boutouyrie, P., Alivon, M., & Reesink, K. D. (2017). Patient-specific blood pressure correction technique for arterial stiffness: evaluation in a cohort on anti-angiogenic medication. *Hypertension Research*, 40(8), 752-757. <https://doi.org/10.1038/hr.2017.32>

Document status and date:

Published: 01/08/2017

DOI:

[10.1038/hr.2017.32](https://doi.org/10.1038/hr.2017.32)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

ORIGINAL ARTICLE

Patient-specific blood pressure correction technique for arterial stiffness: evaluation in a cohort on anti-angiogenic medication

Bart Spronck^{1,2}, Tammo Delhaas¹, Anouk GW De Lepper¹, Julie Giroux³, François Goldwasser³, Pierre Boutouyrie⁴, Maureen Alivon⁴ and Koen D Reesink¹

Arterial pulse wave velocity (PWV) depends on blood pressure (BP). Correction of PWV for BP is commonly performed using a statistical approach, requiring a patient cohort. We recently developed a mechanistic, model-predictive approach to assess BP-independent changes in carotid PWV (cPWV) at the level of the individual. The goal of the present study is to compare our novel technique to conventional statistical correction, in the context of anti-cancer therapy using anti-angiogenic drugs (AADs). AADs frequently lead to a PWV increase, but also to hypertension, underlining the need for BP correction of PWV measurements. We obtained carotid artery systolic and diastolic cross-sectional areas (echotracking) and corresponding BPs (tonometry) in 48 patients before starting AAD treatment (sorafenib/sunitinib), and at four follow-up visits spaced 2 weeks apart. For each patient, we derived cPWV and a baseline single-exponential BP cross-sectional area curve. Based on these baseline curves and follow-up BPs, we predicted cPWV at follow-up due to BP. By comparing predicted and measured cPWVs at follow-up, we assessed the BP-independent cPWV increase. In the same way, we assessed whether diastolic cross-sectional area (A_d) changed beyond the BP-induced amount. The AAD-induced BP-independent increase in cPWV was $0.43(0.09, 0.77) \text{ m s}^{-1}$ (mean (95%CI), $P=0.014$, mechanistic approach) and $0.48(0.14, 0.82) \text{ m s}^{-1}$ ($P=0.006$, statistical approach). A_d increased with $1.92(0.93, 2.92) \text{ mm}^2$ ($P<0.001$) and $2.14(1.06, 3.23) \text{ mm}^2$ ($P<0.001$), respectively. In conclusion, the present study demonstrates the feasibility and potential of our mechanistic, model-predictive approach to quantify BP-independent effects on arterial stiffness at the level of the individual, in a clinically relevant setting of AAD therapy.

Hypertension Research (2017) 40, 752–757; doi:10.1038/hr.2017.32; published online 16 March 2017

Keywords: cancer and stroke; carotid artery; pulse wave velocity; ultrasonography

INTRODUCTION

Anti-angiogenic drugs (AADs) are increasingly used in anti-cancer therapy.¹ We recently showed that AADs lead to an increase in local carotid pulse wave velocity (cPWV),² a measure of large artery stiffening. Furthermore, AAD treatment frequently leads to hypertension.³ Since PWV is known to depend on blood pressure (BP),^{4–7} the increased cPWV does not directly reflect intrinsic large artery stiffening. Therefore, using PWV to assess intrinsic arterial stiffness requires consideration of BP.

In a recent study, we demonstrated that by using distensibility measurements at carotid level and a mechanistic approach, it is possible to assess the BP effect on cPWV in the individual patient, rather than adjusting for BP at the group level.⁸ This approach is based on a single-exponential relationship that is fitted to arterial

pressure–area measurements, and allows estimation of any changes in stiffness due to changes in wall material and not due to BP.

In the present study, we will use our novel mechanistic approach to quantify the BP-independent effect of AADs on large artery stiffness. We will compare this novel mechanistic approach to the conventional, exclusively statistical correction for BP effects, and discuss the differences between these two methods.

METHODS

Study population and measurements

The population and measurements used in this study were elaborately described previously.² Briefly, patients in whom treatment with AADs (sorafenib or sunitinib) was indicated were recruited at a secondary unit dedicated to care of metastatic cancer patients (Figure 1 in Alivon *et al.*²). Patients were investigated during day hospitalisation for chemotherapy administration, at baseline,

¹Department of Biomedical Engineering, CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, The Netherlands; ²Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia; ³Department of Medical Oncology, CERIA, Cochin Hospital, Assistance Publique—Hôpitaux de Paris, Paris, France and ⁴Department of Pharmacology, Université Paris Descartes, European Georges Pompidou Hospital, Assistance Publique—Hôpitaux de Paris, Paris, France

Correspondence: Dr KD Reesink, Department of Biomedical Engineering, CARIM School for Cardiovascular Diseases, Maastricht University, Universiteitssingel 50 (room 3.360), PO Box 616, 6200 MD, Maastricht, The Netherlands.

E-mail: k.reesink@maastrichtuniversity.nl

Received 14 November 2016; revised 12 January 2017; accepted 7 February 2017; published online 16 March 2017

Figure 1 Study set-up. At baseline (top row), subjects were measured before onset of anti-angiogenic drugs (AADs). Directly after the baseline measurement, and before follow-up visits (bottom row) subjects were put on AADs. During both baseline and follow-up visits, measurements (left column) were performed which included carotid artery tonometry and ultrasound wall tracking. These yielded diastolic (DBP, A_d) and systolic (SBP, A_s) pressure–area points (left column). At baseline, an exponential pressure–area curve was modelled through these points (top right) for each subject individually. At follow-up, this (unchanged) curve, together with follow-up pressures, was used to predict diastolic and systolic cross-sectional areas (A_{dpred} and A_{spred} , bottom right). Measured carotid pulse wave velocity (cPWV) was calculated using measured A 's, whereas predicted cPWV ($cPWV_{pred}$) was calculated using predicted A 's (legend). In both cases the Bramwell–Hill equation is used.¹⁷ The difference between measured and predicted cPWVs ($\Delta cPWV$) was calculated by subtracting $cPWV_{pred}$ from cPWV. Similarly, ΔA_d was calculated by subtracting A_{dpred} from A_d . ρ refers to blood mass density; 'area' refers to the artery lumen cross-sectional area.

after 7–10 days of AADs (follow-up 1) and then every 2 weeks for 6 weeks (follow-ups 2–4). At each visit, brachial diastolic (DBP), mean (MAP) and systolic (SBP) BPs were recorded in triplicate using an oscillometric device (Dinamap, GE Medical, Tampa, Florida, USA). If AADs induced hypertension, the consulting physician put patients on anti-hypertensive drugs. Systolic, mean and diastolic pressures were averaged over the three acquired measurements by calculating the average. In addition, carotid artery ultrasonic wall tracking was performed (Artlab; Esaote Pie-Medical, Maastricht, the Netherlands).² Local carotid artery pulse pressure was calculated using the carotid applanation tonometry waveform recorded with a SphygmoCor device (Atcor Medical, Sydney, Australia).² This approach assumes carotid DBP and MAP to equal brachial DBP and MAP.⁹ Patients provided written informed consent before baseline testing. The study protocol was performed within the Angiogenesis Inhibitors Study and Research Centre (CERIA) and was approved by the Cochin Ethics committee (approval number 12804766).

Data quality

Ultrasonic wall tracking as well as tonometry measurements were performed by researchers who had been trained by a highly experienced investigator (PB) for ≥ 2 weeks. This training period was concluded if in a reproducibility study in ≥ 15 patients the short-term coefficient of variation (between investigators) was $< 3\%$ for diameter, $< 6\%$ for distension and $< 4\%$ for PWV. Furthermore, in the present study, quality of the applanation tonometry measurements was assessed using the operator index, only keeping measurements with an operator index > 80 . In two exceptional conditions, measurements with a lower operator index were retained: (1) if the wave shape appeared regular but only the end-diastolic pressure curve showed beat-to-beat variability; or (2) if the low operator index was caused by one outlying beat (an extrasystole).

Data processing

Data processing was performed using the software R, version 3.2.3.¹⁰

Carotid stiffness calculation. Cross-sectional areas of the carotid artery lumen were calculated from the media-adventitia echo diameter using $A = \pi \times (\text{diameter}/2)^2$ at diastole (A_d) and systole (A_s). cPWV was calculated

using the Bramwell–Hill relationship:¹¹

$$cPWV = \sqrt{\frac{1}{\rho} \frac{SBP - DBP}{A_s - A_d} A_d}, \quad (1)$$

with $\rho = 1.050 \text{ kg l}^{-1}$ the blood mass density, and SBP and DBP the systolic and diastolic brachial BPs, respectively.

Uncorrected effect of AADs on BPs, cPWV and A_d . The uncorrected effect of AADs on BPs, cPWV and A_d was assessed using mixed-effects models (R nlme package version 3.1–125)¹² of the form:

$$\text{par} = \beta_0 + \beta_1 \text{AAD}, \quad (2)$$

where par is brachial SBP, carotid SBP, MAP, DBP, cPWV or A_d , respectively. AAD is a Boolean variable indicating the use of AADs, and is therefore coded 0 for the baseline visit and 1 for all follow-up visits. Therefore, β_0 represents the average value of par at baseline, and β_1 represents the average AAD-induced change in par over all follow-up visits.

Mixed-effects modelling has the distinct advantage over, for example, repeated-measures analysis of variance that it can handle missing data points, maximising the use of all available data. Furthermore, mixed-effects modelling has proven to be very robust against non-normality of residuals.¹³ The most appropriate covariance structure was determined for each model by likelihood-ratio comparison of 8 models with different covariance structures.¹⁴ Likelihood-ratio tests were used to compare the different covariance structures.^{14,15} After the most suitable covariance structure was found, significance of fixed model terms was assessed by likelihood-ratio comparison of successively more complicated models.

Mechanistic approach. Pressure–area curve description. The diastolic and systolic pressure–area (P – A) points obtained at baseline (before anti-angiogenic treatment) were used to fit an established mathematical description

of the P - A relation, that is, a single-exponential model:¹⁶

$$P(A) = P_{\text{ref}} \cdot e^{\gamma \left(\frac{A}{A_{\text{ref}}} - 1 \right)}, \text{ or equivalently} \quad (3)$$

$$A(P) = A_{\text{ref}} \left(\frac{\ln \left(\frac{P}{P_{\text{ref}}} \right)}{\gamma} + 1 \right), \quad (4)$$

where P_{ref} and A_{ref} indicate reference diastolic BP and cross-sectional area, respectively. In this study, $P_{\text{ref}} = 100$ mm Hg was chosen.

Model predictions of cPWV and A_d at follow-up. For each individual patient, we predicted A_d ($A_{d,\text{pred}}$) and A_s ($A_{s,\text{pred}}$) at follow-up based on the baseline P - A curves (Equation (4)) and follow-up BPs (Figure 1). Subsequently, predicted cPWV (cPWVpred) was obtained from Equation (1), using follow-up BPs and predicted cross-sectional areas ($A_{d,\text{pred}}$ and $A_{s,\text{pred}}$). This was done under the explicit assumption that between baseline and follow-up the P - A relationship had remained unaltered. Therefore, any difference between measured and predicted cPWV or A_d signifies a change in intrinsic wall properties and is not a BP effect.

Mixed-effects modelling to assess statistical significance of cPWV and A_d predictions. The BP-independent effect of AADs on carotid artery stiffness was analysed by calculating the difference between predicted (cPWVpred) and measured cPWV at each follow-up visit, which was termed Δ cPWV. An initial mixed-effects model with Δ cPWV as dependent variable was fitted to the data:

$$\Delta \text{cPWV} = \beta_0 + \beta_1 d_{n_{\text{visit}},1} + \beta_2 d_{n_{\text{visit}},2} + \beta_3 d_{n_{\text{visit}},3} + \beta_4 \text{AHD}, \quad (5)$$

containing:

1. An intercept (parameter β_0).
2. The visit number as a categorical variable (parameters β_1 - β_3), which was added by means of three dummy variables ($d_{n_{\text{visit}},1}$ - $d_{n_{\text{visit}},3}$), for which deviation coding was used. As there are four follow-up visits, there are three dummy variables.
3. Use of anti-hypertensive drugs (parameter β_4), as a Boolean variable (AHD), which was also deviation-coded.

Baseline data points were not used in fitting the models, since these per definition only contain zeroes (Δ cPWV = 0 at baseline) and lead to numerical problems in model fitting. Notably, the use of deviation coding for the categorical and Boolean variables ensures that the model's intercept term corresponds to the grand mean of the model. Therefore, in the current formulation, a (positive) significant intercept term indicates that Δ cPWV is significantly larger than 0. The latter implies that measured cPWV is significantly larger than predicted cPWV, indicating a BP-independent increase in cPWV at follow-up. Using the fixed model terms as described in Equation (5), the most appropriate covariance structure was determined.^{14,15}

Difference between A_d prediction and measurement. The BP-independent effect of AAD treatment on carotid diastolic cross-sectional area ($\Delta A_d = A_d - A_{d,\text{pred}}$) was analysed using the same scheme as for the Δ cPWV analysis (see above).

Conventional, entirely statistical approach. Correcting for the BP dependency of cPWV. Conventionally, PWV is corrected for BP using a statistical model. In the present study, we replicated such an approach. We fitted the following initial mixed-effects model to the data:

$$\text{cPWV} = \beta_0 + \beta_1 \text{AAD} + \beta_2 \text{DBP} + \beta_3 \text{DBP}^2 + \beta_4 d_{n_{\text{visit}},1} + \beta_5 d_{n_{\text{visit}},2} + \beta_6 d_{n_{\text{visit}},3} + \beta_7 \text{AHD} \quad (6)$$

In contrast to the mixed-effects models used in our mechanistic approach, cPWV (Equation (6)) is fitted to all five visits, including the baseline visit. Note that the dependent parameter is now cPWV instead of Δ cPWV as in Equation (5). AAD is a Boolean variable indicating the use of AADs, and is therefore coded 0 for the baseline visit and 1 for all follow-up visits. Note that

while each patient has five visits, there are only three visit dummy parameters present. This is necessary, as AAD also functions as a visit dummy variable, effectively distinguishing between baseline and follow-up visits. For the coding of AAD and $d_{n_{\text{visit}},1}$ - $d_{n_{\text{visit}},3}$, see Supplementary Table S4. The most suitable covariance structure was again estimated from eight potential candidates.¹⁴

Correcting for the BP dependency of A_d . Exactly the same, entirely statistical approach that was used to estimate the BP-independent effect of AADs on cPWV (see previous paragraph) was used to estimate the BP-independent effect of AADs on A_d .

RESULTS

Patient population

The same patient data as in Alivon *et al.*² were used. In the present study, only subjects with complete baseline measurements (echotracking, carotid tonometry and BP; see below) were included ($n = 48$). General characteristics of this group are outlined in Table 1. At the follow-up visits, $n = 39$ (follow-up 1), $n = 30$ (follow-up 2), $n = 31$ (follow-up 3), and $n = 23$ (follow-up 4) measurements were included.

Uncorrected effects of AADs on BPs, cPWV and A_d

Table 2 shows the estimated, uncorrected effects of AADs on BPs, cPWV, and A_d . AADs increased cPWV and A_d by 0.75 m s^{-1} and 2.7 mm^2 on average, respectively (both $P < 0.001$). However, all BP measures also significantly increased ($P \leq 0.006$). This could potentially explain the increased cPWV and A_d that were measured, and emphasises the need for a method to correct for BP. Heart rate was not influenced by AADs ($P = 0.602$).

Main findings of mechanistic and conventional, statistical BP correction approaches

Using our mechanistic approach, for each subject, the difference between measured and predicted cPWV (Δ cPWV) was calculated. For the predicted cPWV values, strictly no change in wall behaviour is assumed, that is, the P - A relationship remains unaltered. All differences between measured and predicted cPWVs are therefore assumed to be caused by intrinsic wall changes. Mixed-effects modelling was used to investigate the statistical significance of Δ cPWV. We found that AADs lead to a BP-independent increase in Δ cPWV of $0.43(0.09,0.77) \text{ m s}^{-1}$ ($P = 0.014$, Table 3). The change in cPWV was also assessed by an exclusively statistical approach, which resulted in a cPWV change of $0.48(0.14,0.82) \text{ m s}^{-1}$ ($P = 0.006$, Table 3).

Our mechanistic approach was also used to investigate the BP-independent effect of AADs on diastolic cross-sectional area (A_d). We found that AADs lead to an increase in ΔA_d of 1.92

Table 1 Study population baseline characteristics

Parameter	Units	n = 48
Sex	n (male/female)	30/18
Age	years	56 ± 15
Height	m	1.72 ± 0.11
Weight	kg	75 ± 13
BMI	kg m^{-2}	25 ± 4
Hypertension	n (yes/no)	16/32
Dyslipidemia	n (yes/no)	14/34
Diabetes	n (yes/no)	5/43

Abbreviation: BMI, body mass index.
Mean $\pm$ s.d. unless otherwise indicated.

(0.93,2.92) mm² ($P<0.001$, Table 3). Using the exclusively statistical approach, this change was estimated at 2.14(1.06,3.23) mm² ($P<0.001$, Table 3).

Mechanistic approach: changes in intrinsic carotid artery stiffness with the use of anti-angiogenic medication

Supplementary Table S1 contains the full analysis results discussed in this section. Mixed-effects modelling was used to investigate the effect of AADs on Δ cPWV, as well as the potential effects of anti-hypertensive medication and the potential difference in Δ cPWV between follow-up visits. Model 1 in Supplementary Table S1 (of which β_0 is reproduced in Table 3) represents the mixed-effects model with the simplest fixed-effects structure, that is, only an intercept. The magnitude of the intercept term indicates that measured cPWVs are on average 0.43 m s⁻¹ higher than predicted cPWVs, at $P=0.014$. Addition of other model terms (distinguishing between follow-up visits and/or between the use of anti-hypertensive medication) did not significantly improve the statistical model (Supplementary Table S1, right column: likelihood-ratio tests are all non-significant).

Mechanistic approach: changes in intrinsic carotid artery diameter with the use of anti-angiogenic medication

Supplementary Table S2 contains the full analysis results discussed in this section. Again, for the predicted A_d values, strictly no change in wall behaviour is assumed. The same mixed-effects modelling approach as for cPWV was used. Model 1 in Supplementary Table S2 (of which β_0 is reproduced in Table 3) represents the mixed-effects model with the simplest fixed-effects structure, that is, only an intercept. The magnitude of the intercept term indicates that measured A_d s are on average 1.9 mm² higher than predicted from the BP increase, at $P<0.001$. Addition of other model terms (distinguishing between follow-up visits and/or between the use of anti-hypertensive medication) again did not significantly improve the statistical model.

Table 2 Estimated first-order effects of AADs on BPs, cPWV and A_d

Parameter	Units	Baseline	Change at follow-up	P-value
SBP, brachial	mm Hg	123.2 (118.0,128.4)	8.3 (4.6,12.0)	<0.001
SBP, carotid	mm Hg	117.1 (111.1,123.1)	8.6 (3.7,13.4)	<0.001
MAP	mm Hg	92.7 (88.1,97.2)	5.3 (1.6,9.1)	0.006
DBP	mm Hg	73.3 (70.1,76.5)	6.2 (4.0,8.5)	<0.001
cPWV	m s ⁻¹	6.82 (6.39,7.26)	0.75 (0.52,0.98)	<0.001
A_d	mm ²	42.0 (38.9,44.9)	2.7 (1.7,3.6)	<0.001
HR	b.p.m.	75.3 (71.3,79.2)	-0.9 (-4.1,2.4)	0.602

Abbreviations: AAD, anti-angiogenic drug; A_d , diastolic carotid lumen cross-sectional area; BP, blood pressure; cPWV, local carotid pulse wave velocity; DBP, diastolic BP; HR, heart rate; MAP, mean arterial pressure; SBP, systolic BP.

Mean (95% confidence interval) values of mixed-effects model (Equation (2)) intercept term (β_0 , baseline) and AAD term (β_1 , change at follow-up). Note that the changes in cPWV and A_d presented in this table have not been corrected for BP effects yet.

Table 3 Summary of BP-independent effects of AADs on cPWV and A_d

Mechanistic correction				Exclusively statistical correction			
Effect of AADs (β_0)		Reference		Effect of AADs (β_1)		Reference	
cPWV (m s ⁻¹)	0.43 (0.09,0.77)	$P=0.014$	Supplementary Table S1, #1	0.48 (0.14,0.82)	$P=0.006$	Supplementary Table S5, #5	
A_d (mm ²)	1.92 (0.93,2.92)	$P<0.001$	Supplementary Table S2, #1	2.14 (1.06,3.23)	$P<0.001$	Supplementary Table S6, #3	

Abbreviations: AAD, anti-angiogenic drug; A_d , diastolic carotid lumen cross-sectional area; BP, blood pressure; cPWV, local carotid pulse wave velocity.

Summary of BP-independent effects of AADs on cPWV and A_d , as assessed by our novel mechanistic correction approach (left part of table) and by an exclusively statistical approach (right part of table). Values are given as mean (95% confidence interval).

Changes in diastolic BP with the use of anti-angiogenic medication

Supplementary Table S3 contains the full analysis results discussed in this section. As an internal check, we assessed whether DBP did indeed increase with AAD, and whether this increase differed between follow-up visits and between people that did or did not use anti-hypertensive drugs. Supplementary Table S3 shows mixed-effects models comparing DBP at each of the follow-up visits to baseline. Model #1 shows that at the follow-up visits, DBP was 6.0 mm Hg higher than at baseline ($P<0.001$). Addition of other model terms (distinguishing between follow-up visits and/or between the use of anti-hypertensive medication) did not significantly improve the statistical model.

Conventional, entirely statistical approach: correcting for the BP dependency of cPWV

Supplementary Table S5 contains the full analysis results discussed in this section. In addition to our novel mechanistic methodology, we assessed the effects of AADs on cPWV by means of a statistical approach, without prediction of follow-up cPWVs. Supplementary Table S5 shows the results of a series of mixed-effects model fits. As expected, DBP had a significant influence on cPWV (addition of a DBP term improved the model, $P=0.010$, model #2 vs. #1). Additional inclusion of a quadratic DBP term did not statistically significantly improve the model ($P=0.288$, model #3 vs. #2). Nevertheless, we chose to continue statistical modelling with (models #5 and #7) and without (models #4 and #6) a quadratic DBP term. Distinguishing between visits improved the model ($P=0.002$, model #4 vs. #2 and $P=0.003$, model #5 vs. 3). Addition of a term accounting for the presence of anti-hypertensive medication did not improve the model, whether it was to a model without a quadratic DBP term ($P=0.468$, models #6 vs. #4), or to a model with a quadratic DBP term ($P=0.568$, models #7 vs. #5).

From a strictly statistical point of view (only keeping model terms that significantly improve the statistical model), model #4 (Supplementary Table S5) best describes our results. This model indicates an AAD-induced increase in cPWV of 0.52 m s⁻¹. However, as there is a known nonlinear dependence of cPWV on DBP, using a model that corrects for DBP quadratically provides additional BP correction (model #5, of which β_1 is reproduced in Table 3). This model indicates an AAD-induced increase in cPWV of 0.48 m s⁻¹.

Conventional, entirely statistical approach: correcting for the BP dependency of A_d

Supplementary Table S6 contains the full analysis results discussed in this section. Supplementary Table S6 shows the results of a series of mixed-effects model fits that assess the AAD-induced change in A_d on a statistical basis. The influence of DBP on A_d is statistically non-significant ($P=0.105$, model #2 vs. #1). However, there is a clear, direct, mechanical relationship between pressure and lumen cross-sectional area. Addition of a quadratic term is again statistically

non-significant ($P=0.168$, model #3 vs. #2), albeit that physiologically, the relationship between A_d and pressure is known to be nonlinear (see, for example, Equation (3)). We have chosen to continue our statistical modelling again on 'physiological grounds', keeping in both the DBP and DBP² terms (models #5 and #7), as well as performing parameter inclusion strictly statistically, omitting DBP terms altogether (models #4 and #6). Neither distinguishing between visits ($P=0.553$, model #4 vs. #1 and $P=0.673$, model #5 vs. #3) nor accounting for the presence of anti-hypertensive medication ($P=0.584$, model #6 vs. #1 and $P=0.584$, model #7 vs. #3) improved our models.

From a strictly statistical point of view (only keeping model terms that significantly improve it), model #1 (Supplementary Table S6) best describes our results. This model indicates an AAD-induced, BP-corrected increase in A_d of 2.67 mm^2 . However, to obtain a physically warranted BP correction, a model should be used that corrects for the nonlinear dependence of A_d on DBP (model #3, of which β_1 is reproduced in Table 3). The latter model indicated an AAD-induced, BP-corrected increase in A_d of 2.14 mm^2 .

DISCUSSION

In the present study, we investigated the effect of AADs on arterial stiffness as quantified by cPWV. As cPWV is known to vary heavily with BP,^{5–7,17} one has to correct cPWV for this potential confounder. Recently, we published a study in which we quantified the BP effect on cPWV on a mechanistic basis, and proposed a way of correcting for this effect.⁸ We applied this mechanistic correction approach to the data in the present study, and found that AAD treatment leads to a BP-independent increase in cPWV of 0.43 m s^{-1} . With AAD treatment, arterial lumen cross-sectional area showed a BP-independent increase of 1.9 mm^2 . We compared these findings obtained using our mechanistic approach to the results of the conventional approach of statistical correction. When correcting for a quadratic dependence of cPWV and A_d on DBP, we found an AAD-induced increase of 0.48 m s^{-1} in cPWV and 2.1 mm^2 in A_d . These numbers are similar to those obtained using our mechanistic approach, confirming that our mechanistic, model-driven methodology yields BP-corrected estimates of cPWV and A_d that are very similar to their statistically predicted counterparts. The advantage of our mechanistic methodology, however, is that it provides a pressure-independent estimation of cPWV and A_d at the level of the individual, which is crucial for clinical management.

In our previous paper,⁸ we used three pressure–area points (diastolic, dicrotic notch and systolic) to fit the curvilinear relationship between pressure and area. In the present study, dicrotic notch detection in the pressure and diameter signals was unavailable due to technical limitations, and therefore we resorted to a two-point approach. Our results show that the pressure dependence of cPWV can also be reasonably captured using a two-point approach, although we could not establish the possible quantitative consequences in the present study.

While mean arterial pressure is often used in correcting for BP, from a physical point of view, diastolic BP may be the actual parameter that is the main determinant of the velocity of the BP wave.^{4,18} We therefore chose to present findings based on statistical correction using diastolic BP in the main text of this paper. As the choice between diastolic and mean pressure for correcting PWV is subject of ongoing discussion, we have also performed statistical BP corrections based on mean arterial pressures. Results from these analyses are presented in Supplementary Digital Content 1, Supplementary Tables S7 and S8, and lead to an AAD effect on cPWV and A_d of 0.46 m s^{-1} and 2.3 mm^2 , respectively. These results

differ only minimally from statistical results obtained using DBP as correction variable.

In our approach, predictions of cPWV and A_d at all follow-up visits are based on the baseline P – A curve. This renders subsequent measurements dependent on the baseline value. P – A curves were based on one diastolic and one systolic P – A point. Therefore, any noise in the baseline measurements will have direct effects on the calculated predictions. We also established an average P – A curve for each subject along all visits, and performed predictions using these average curves instead of baseline curves. This approach is elaborated in Supplementary Digital Content 2. Estimated, BP-independent changes in cPWV and A_d were 0.25 m s^{-1} and 1.92 mm^2 , respectively. These numbers are lower than those from our baseline curve-based method. This difference is caused by the statistical modelling approach that was chosen to estimate the most appropriate covariance structure (for details, see ¹⁴). In the method based on average P – A curves, the baseline visit is also included in the model. This led to a more complicated covariance structure than in the original method, which reduced the magnitude of the estimated fixed effect of anti-angiogenic treatment.

Instead of calculating differences between measured and predicted cPWVs and A_d s, one can also directly assess the parameters γ and A_{ref} of the P – A curves. Results of these analyses are presented in Supplementary Digital Content 2. The increase in γ with anti-angiogenic treatment confirms the increase in pressure-independent stiffness as quantified by ΔcPWV ; while the increase in A_{ref} confirms the pressure-independent increase in diameter. The advantage of this method is that γ and A_{ref} are directly pressure-independent, and no calculation of a difference between prediction and measurement is necessary. However, ΔcPWV gives the user a direct estimate of the stiffness change in cPWV units (that is, in metres per second), enabling direct comparison of the magnitude of ΔcPWV to absolute cPWV.

The structural changes underlying the increased stiffness and cross-sectional area of the carotid artery wall with AAD use are largely unknown. Several potential causes are discussed in our previous paper,² which include potential vasoactive properties of sorafenib and sunitinib¹⁹ or their interaction with integrins, but also a potential effect of the AADs on the vasa vasorum, the microscopic arterial network that supply the artery wall with nutrients.²⁰ It is beyond the scope of the present study to structurally explain the observed changes in carotid artery stiffness and diameter.

The carotid BP measurements used in this study are obtained by scaling the carotid artery applanation tonometry waveform, assuming that carotid diastolic and mean pressures are equal to brachial diastolic and mean pressures.⁹ This scaling method has two potential disadvantages: (1) it introduces additional measurement noise, and (2) it requires additional tonometry measurements by an experienced research nurse,²¹ complicating the measurement protocol. In addition to the present analyses, we additionally re-performed our mechanistic correction technique using brachial systolic and diastolic pressures. All results were essentially the same, except for the observation that the pressure-independent increase in ΔcPWV with AAD got smaller and lost statistical significance ($\beta_0=0.314 \text{ m s}^{-1}$, $P=0.075$). This suggests that using brachial pressures instead of carotid yields an under-estimation of ΔcPWV .

The technique presented in this study can not only be applied to local cPWV, but also to carotid-femoral transit time PWV (cfPWV). Although with cfPWV the pressure–area relationship is not directly measured, the exponent of this relationship can be directly calculated from cfPWV and BP measurements. Using this exponent, cfPWV

predictions based on BP changes can be made in the same way as for cPWV, and $\Delta cPWV$ (the difference between measured and predicted cPWV) can also be calculated, underlining the potential of our mechanistic method.

From the clinical perspective, cPWV as commonly obtained depends on BP, and can therefore not be used as an independent measure of intrinsic arterial stiffness. This BP dependence cannot be corrected for in a straightforward manner. In population studies, correction can be performed statistically. However, this requires a patient cohort for the statistical model to be quantified. Our study demonstrates that cPWV can also be corrected for BP in a mechanistic way. This method is applicable in individual patients, and can be used to assess an individual's change in intrinsic arterial stiffness over time, independent of BP, and without the subject being part of a study cohort.

In conclusion, the present study demonstrates the feasibility and potential of our mechanistic, model-predictive approach to quantify BP-independent effects on arterial stiffness at the level of the individual, in a clinically relevant setting of AAD therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

BS was supported by a Kootstra Talent Fellowship awarded by Maastricht University and by an Endeavour Research Fellowship awarded by the Australian Government.

- 1 Cao Y, Langer R. Optimizing the delivery of cancer drugs that block angiogenesis. *Sci Transl Med* 2010; **2**: 15ps13.
- 2 Alivon M, Giroux J, Briet M, Goldwasser F, Laurent S, Boutouyrie P. Large artery stiffness and hypertension after antiangiogenic drugs: influence on cancer progression. *J Hypertens* 2015; **33**: 1310–1317.
- 3 Wu S, Chen JJ, Kudelka A, Lu J, Zhu X. Incidence and risk of hypertension with sorafenib in patients with cancer: a systematic review and meta-analysis. *Lancet Oncol* 2008; **9**: 117–123.

- 4 Bramwell JC, McDowall RJS, McSwiney B. The variation of arterial elasticity with blood pressure in man (part I). *Proc R Soc Lond B* 1923; **94**: 450–454.
- 5 Stepan J, Sikka G, Hori D, Nyhan D, Berkowitz DE, Gottschalk A, Barodka V. Seeking a blood pressure-independent measure of vascular properties. *Hypertens Res* 2016; **39**: 27–38.
- 6 Schillaci G, Battista F, Settimi L, Anastasio F, Pucci G. Cardio-ankle vascular index and subclinical heart disease. *Hypertens Res* 2015; **38**: 68–73.
- 7 Spronck B, Avolio AP, Tan I, Butlin M, Reesink KD, Delhaas T. Arterial stiffness index beta and cardio-ankle vascular index inherently depend on blood pressure but can be readily corrected. *J Hypertens* 2017; **35**: 98–104.
- 8 Spronck B, Heusinkveld MH, Vanmolkot FH, Roodt JO, Hermeling E, Delhaas T, Kroon AA, Reesink KD. Pressure-dependence of arterial stiffness: potential clinical implications. *J Hypertens* 2015; **33**: 330–338.
- 9 Van Bortel LM, Balkestein EJ, van der Heijden-Spek JJ, Vanmolkot FH, Staessen JA, Kragten JA, Vredeveld JW, Safar ME, Struijker Boudier HA, Hoeks AP. Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. *J Hypertens* 2001; **19**: 1037–1044.
- 10 R Core Team. *R: A language and Environment for Statistical Computing*. Available at <http://www.R-project.org/>. Accessed on 11 March 2016.
- 11 Bramwell JC, Hill AV. The velocity of the pulse wave in man. *Proc R Soc B* 1922; **93**: 298–306.
- 12 Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. *Nlme: Linear and Nonlinear Mixed Effects Models*. Available at <http://cran.r-project.org/package=nlme>. Accessed on 11 March 2016.
- 13 Gelman A, Hill J. *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press, Cambridge, UK, 2007.
- 14 Walavalkar V, Evers E, Pujar S, Viralam K, Maiya S, Frerich S, John C, Rao S, Reddy C, Spronck B, Prinzen FW, Delhaas T, Vanagt WY. Preoperative sildenafil administration in children undergoing cardiac surgery: a randomized controlled preconditioning study. *Eur J Cardiothorac Surg* 2015; **49**: 1403–1410.
- 15 Zuur AF. *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY, USA, 2009.
- 16 Meinders JM, Hoeks AP. Simultaneous assessment of diameter and pressure waveforms in the carotid artery. *Ultrasound Med Biol* 2004; **30**: 147–154.
- 17 Bramwell JC, Hill AV. The velocity of the pulse wave in man. *Proc R Soc Lond B* 1922; **93**: 298–306.
- 18 Nichols WW, Nichols WW, McDonald DA. *McDonald's Blood Flow in Arteries: Theoretic, Experimental, and Clinical Principles*. CRC Press, London, UK, 2011.
- 19 Papadopoulou DN, Mendrinou E, Mangioris G, Donati G, Pournaras CJ. Intravitreal ranibizumab may induce retinal arteriolar vasoconstriction in patients with neovascular age-related macular degeneration. *Ophthalmology* 2009; **116**: 1755–1761.
- 20 Stefanadis C, Vlachopoulos C, Karayannacos P, Boudoulas H, Stratos C, Filippides T, Agapitos M, Toutouzas P. Effect of vasa vasorum flow on structure and function of the aorta in experimental animals. *Circulation* 1995; **91**: 2669–2678.
- 21 Spronck B, Delhaas T, Op 't Roodt J, Reesink KD. Carotid artery applanation tonometry does not cause significant baroreceptor activation. *Am J Hypertens* 2016; **29**: 299–302.

Supplementary Information accompanies the paper on Hypertension Research website (<http://www.nature.com/hr>)