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Running title

Venous pulse wave velocity is affected by PLR

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Abstract

Purpose: The evaluation of a mini or simulated fluid challenge is still a complex and open issue in the clinical setting and it is of paramount significance for the fluid therapy optimization. We here investigated the capacity of a new hemodynamic parameter, the venous Pulse Wave Velocity (vPWV), to detect the effect of passive leg raising (PLR).

Materials and methods: In 15 healthy volunteers (7M, 8F, age 26±3) venous pressure pulses were elicited by pneumatic compressions of the left hand and proximally detected by ultrasound for calculation of the vPWV. We also non-invasively measured the basilic vein (BV) cross-sectional perimeter, and peripheral venous pressure (PVP). The PLR manoeuvre was performed twice to evaluate reliability of the assessment.

Results: The PLR had an overall statistically significant effect on the entire set of variables (MANOVA, $p<0.05$): vPWV increased from 2.11 ± 0.46 to 2.30 ± 0.47 m/s ($p=0.01$; average increase: 10 %). This effect was transient and dropped below 5 % after about 3 min. A significant increase was also exhibited by BV size and PVP. In consecutive measurements vPWV showed little intra-subject variability (CoV=8%) and good reliability (ICC=0.87). Finally, the vPWV responses to the two PLRs exhibited good agreement (paired T-test: $p=0.96$), and moderate reliability (ICC=0.57).

Conclusion: These results demonstrated that vPWV can be non-invasively, objectively and reliably measured in healthy subjects and that it is adequate to detect small pressure/volume variations, as induced by PLR-from-supine. These characteristics make it suitable for clinical applications.

Keywords: passive leg raising; venous return; vessel stiffness; volume status

Abbreviations:

ANOVA	Analysis of Variance
BSL	Baseline
BV	Basilic vein
CoV	Coefficient of variation
ECG	Electrocardiogram
HR	Heart rate
ICC	Intraclass correlation coefficient
MANOVA	Multivariate analysis of variance
PLR	Passive leg raising
PVP	Peripheral venous pressure
PWV	Pulse wave velocity
STD	Standard deviation
vPWV	Venous pulse wave velocity

1 Introduction

In the last decade, the hemodynamics of the venous compartment has begun to receive more and more attention from the medical community, for its fundamental role in maintaining the cardiovascular equilibrium for correct tissue perfusion [1–3]. Since veins are compliant vessels and host 70% of the total blood volume, an albeit small modulation of such big capacity has the potential of redistributing significantly the blood volume among the body compartments, in particular from/to the splanchnic circulation [4]. This possibility has stimulated the interest of intensivists. Indeed, the patient haemodynamic status assessment for optimal fluid management is nowadays a critical and debated problem of paramount importance in intensive care units' everyday life [5,6] and becomes even more complicated when dealing with patients without catheterization. Despite some recent progress regarding the assessment of fluid responsiveness [7,8], an observational study [9] has shown that its clinical relevance is still underestimated and the commonly adopted procedures are often obsolete. For these reasons, during the last years, a lot of effort has been put in developing non-invasive methods, shifting the paradigm from static to dynamic measurements [8,10]. For instance, the hemodynamic transients associated with respiratory activity have been exploited to assess the respiratory variations in pulse pressure, stroke volume and inferior vena cava diameter. However, all these indicators suffer from specific limitations, due to the inherent variability in the respiratory pattern and appeared to be poor predictors of fluid responsiveness in spontaneously breathing patients [10,11]. Compared to spontaneous breathing, passive leg raising (PLR) appears to produce a more reliable hemodynamic perturbation whose response is normally assessed by continuous cardiac output monitoring [12,13], which however is not commonly available [9]. Irrespectively of the adopted monitoring techniques, the emergent concept regarding fluid responsiveness is the willingness to abandon the dichotomous way of thinking (i.e., classifying patients as responders and non-responders) in favour of a continuous classification of the patient haemodynamic status [8,14],

possibly integrating more than a single parameter. Based on the above considerations, there is a need for additional indicators of current volume status and of fluid responsiveness.

In line with the above considerations, we here explore a novel approach to the characterization of the venous compartment, potentially adequate to provide additional indications on the patient haemodynamic status [15,16], based on the assessment of a novel haemodynamic parameter: the venous Pulse Wave Velocity (vPWV). The PWV is generally measured in arteries as a widely adopted marker of cardiovascular health, being directly proportional to the vessel stiffness [17–19]. Such relation is potentially exploitable also in veins but with two important differences: 1) the lack of a natural pulsatility in venous blood pressure, which may require that artificial pulses are generated and 2) the low venous pressure, which can be easily disturbed by respiratory as well as cardiac activity. These limitations likely discouraged the investigation of vPWV although it was already shown to be linearly dependent on venous pressure [20–23] and sensitive to blood volume losses [24]. An experimental methodology was recently developed which addresses the above-mentioned limitations by artificially generating a venous pressure pulse at a limb extremity with a pneumatic cuff synchronized with the respiratory cycle, the generated pulse wave being then proximally detected by Doppler Ultrasound [25]. Promising results showed that large (8 - 26 mmHg) changes in leg venous pressure consistently produced proportional changes in leg vPWV (1.78 - 2.26 m/s). However, whether this assessment is sensitive enough to detect mild hemodynamic challenges has never been explored in humans.

Thus, this study aimed at assessing whether and to what extent the vPWV responds to a simulated fluid challenge, as provoked by the PLR manoeuvre.

Because of methodological constraints, the PLR had to be conducted from the supine position, thus producing an even smaller hemodynamic challenge than the PLR from the semi-recumbent position [26]. However, compared to our previous study, the stability of the measurement was further improved by synchronizing the measurement non only with the respiratory but also with the cardiac activity. To get an indication of the reliability of the vPWV response, the manoeuvre was repeated

twice on each subject. The perimeter of the cross-sectional area of the insonated vein and the peripheral venous pressure were non-invasively assessed as individual quantitative indicators of the magnitude of the fluid challenge.

2 Materials and Methods

2.1 Subjects

The experiment was conducted on 15 healthy volunteers (7 M, 8 F, age 26 ± 3) with no exclusion criteria. The study was approved by the ethics committee of the University of Torino (March 23, 2015) and all participants gave their informed consent according to the principles of the Helsinki Declaration.

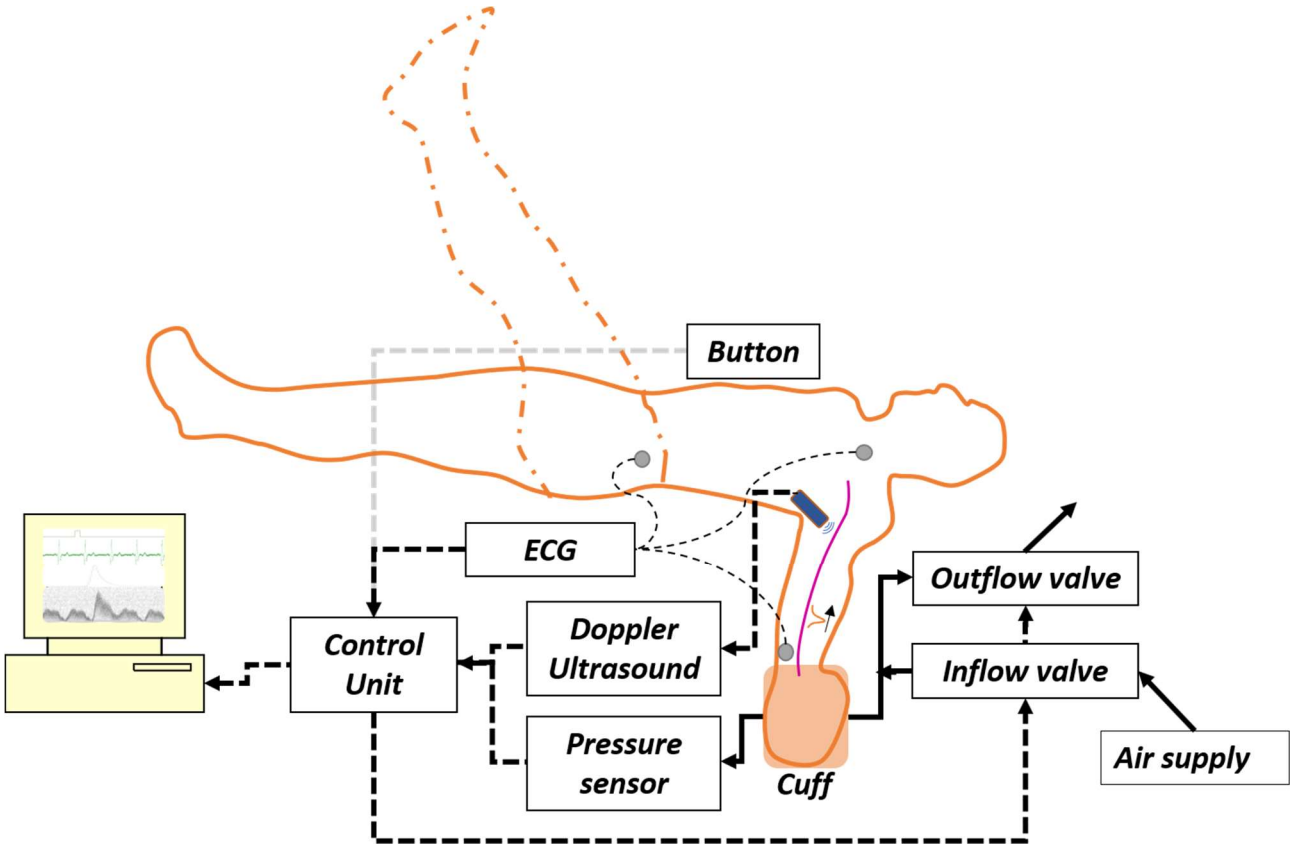

Fig. 1. *Experimental set-up: electrical and pneumatic connections are indicated by dashed and solid lines, respectively*

2.2 Experimental set-up

As anticipated in the introduction, a system was devised for the measurement of vPWV along the arm, based on 1) generation of a pressure wave in venous blood by means of a rapid compression of the hand, 2) synchronous delivery of this compression with respiratory and cardiac activity, 3) proximal detection of the propagated pressure wave by Doppler ultrasound and calculation of the vPWV. Changes in vPWV will be sought during PLR, as compared to the supine position. An overview of the experimental set-up is given in Fig. 1. A pneumatic cuff (49 x 15 cm, GIMA, Gessate, Italy), wrapped around subject's hand, is employed to deliver rapid compressive stimuli to the hand (peak pressure: 400 mmHg, duration: ~1 sec, inflation time: 400 ms). This is achieved by a custom PC-controlled system, previously developed for the investigation of the compression-induced rapid dilatation in skeletal muscles [27,28], composed by a compressed air supply (1 bar) and by two, digitally controlled, electro-pneumatic valves (VXE2330-02F-6D01, SMC, Tokyo, Japan), for inflation and deflation of the cuff. A hand sized hot water bag (filled with water at about 40 °C) was placed on the palm of the hand in order to 1) keep the hand warm and well perfused and 2) permit effective wrapping and compression by the cuff. The hand compression generates a pressure pulse that propagates proximally along venous vessels its passage being monitored by Doppler ultrasound (MyLab 25 Gold, ESAOTE, Genova, Italy) equipped with a linear probe (LA523, ESAOTE, Genova, Italy), at the level of the basilic vein (BV), distally to the armpit, with a transversal approach and an incident angle of about 60 deg [29].

Since venous blood flow and pressure may be affected by both respiratory and cardiac activity, the measurements were always performed 1) at the end of the expiratory phase, that is the most reproducible respiratory position (functional residual capacity) [30], and 2) at the same time position within the cardiac cycle: the one corresponding to the lowest blood velocity (which allowed better detection of the pulse wave).

To this aim, the subject was asked to signal the end of expiration by pressing a hand-held button. After the button was pressed, the occurrence of an R-wave was detected on the ECG (recorded by a

Grass Physiodata Amplifier Model 15LT, Astro-Med Inc., West Warwick, USA), according to a threshold crossing criterion. The pneumatic compression was then started after adding a further adjustable delay of 0-800 ms from the R-wave detection. Such delay was individually set, after few preliminary trials, at the beginning of each experiment to locate the Doppler-detected pulse wave at the point where blood velocity in the BV exhibited the minimum value, within the cardiac cycle (Fig. 2). In fact, the superposition of the generated pulse wave with the spontaneous oscillations of cardiac origin of the venous blood flow could affect the correct detection of the pulse footprint. By adding this further delay to the detection time of the R-wave it was possible to locate the footprint in-between cardiac fluctuations, thus optimizing its detection.

Finally, the cuff pressure was continuously monitored (to locate, precisely in time, the beginning of the compressive stimulus) by a pressure sensor placed at the cuff outlet (Pressure monitor BP-1, WPI, Sarasota, FL, USA) and digitally recorded (Micro 1401 IImk, CED, Cambridge, UK, with Spike2 software), along with the Doppler audio signal, the ECG and the digital signal from the handheld start button, operated by the subject. The same digital board (Micro 1401 IImk) was also used to drive the two electro-pneumatic valves, which were responsible for the compressive stimuli delivery.

2.3 Vessel size and peripheral venous pressure

The cross-sectional perimeter of the BV was calculated from a transversal echographic scan in B-mode, the linear probe oriented at 90 deg with respect to the vein axis. The blood pressure in the BV (PVP, Peripheral Venous Pressure, in mmHg) was estimated as the hydrostatic load relative to the vertical distance (*vd*, in cm) between the venous point of collapse [31,32], i.e., the point in which venous pressure approaches 0 mmHg, and the mid-height of the chest along the anteroposterior direction: PVP = 1.05 * 1.36 * *vd*. The venous point of collapse was echographically sought along the BV of the right arm, which was transiently and passively raised vertically to this purpose. The

venous point of collapse was visualized with a second dedicated ultrasound machine (Mylab 25 XView, Esaote, Genova, Italy, with linear array LA 523).

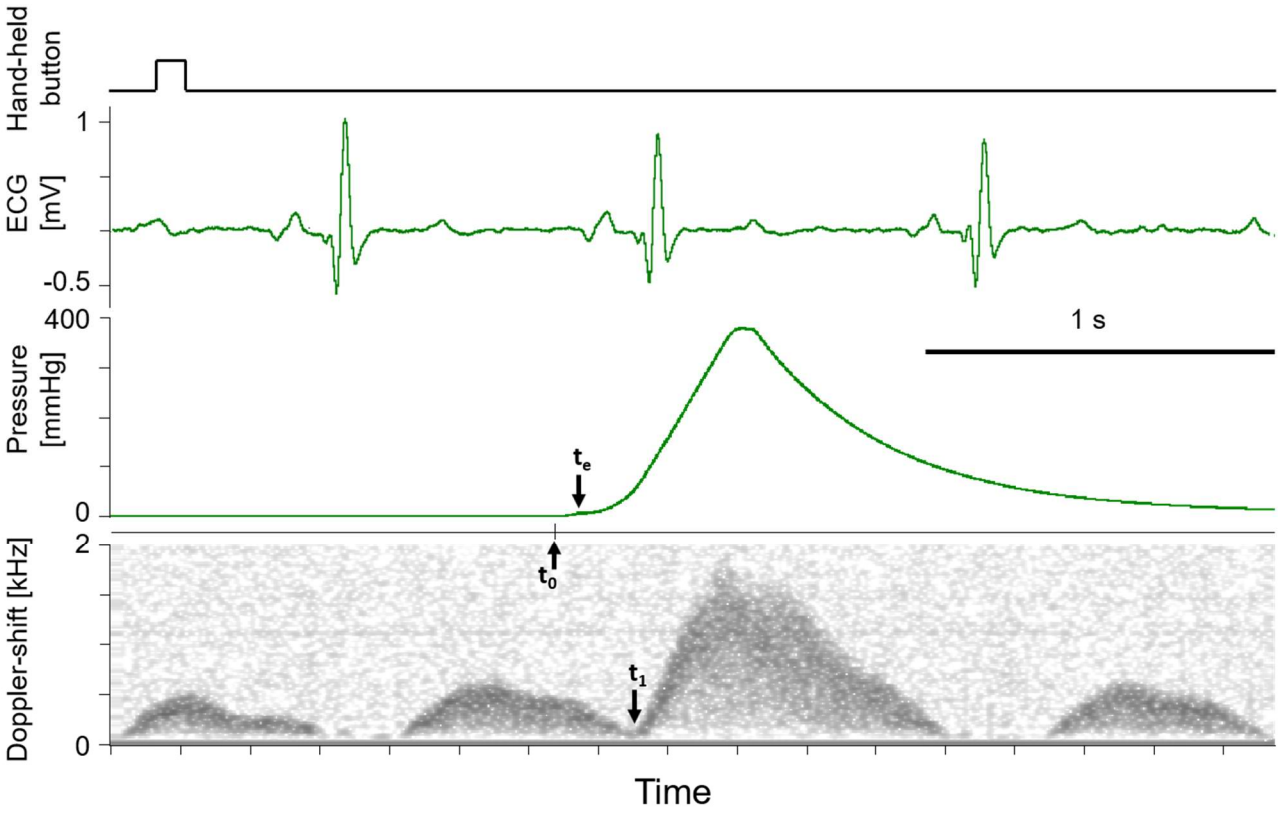

Fig. 2 *Illustration of the synchronization process. From top to bottom: signal from the hand-held button, electrocardiogram, cuff pressure and Doppler shift from the ultrasound monitoring of blood velocity in basilica vein. The button is pressed by the subject at the end of expiration; the algorithm then detects the first R-wave on the ECG trace and after a pre-set delay, opens the inflation valve at t0, detects the beginning of cuff inflation at te, and of the passage of the pulse wave at t1*

2.4 Experimental protocol

The subject remained supine for at least 30 min [30,33] before starting the experimental protocol: two PLR manoeuvres were performed, PLR1 and PLR2, each lasting about 5 min and separated by 8-min rest in the supine position. The PLR was performed by an operator with the help of a pulley, raising and maintaining the extended legs at an angle of about 45 deg. A series of 8 pneumatic compressions were delivered to the hand during both PLRs (PLR1 and PLR2) and baselines i.e., the 5-min intervals preceding each PLR (BSL1 and BSL2), a vPWV measurement being performed for each pulse. In

addition, measurements of BV diameter and peripheral venous pressure were also performed in all conditions.

2.5 Data analysis

The Doppler audio signal was sampled at a rate of 10 kHz and it was exported from Spike2 to Matlab for off-line analysis: a custom-made algorithm was developed to compute the time-domain envelope and to identify the footprint of that profile [17]. As first step, the relevant epochs of 1-s width, starting from the time (t_0) at which the control unit delivered the trigger for cuff inflation, were identified (Fig. 2). Then, the signal was digitally band-pass filtered between 100 and 2000 Hz (approximately equivalent to 3-60 cm/s in terms of blood velocity), after noticing, by visual inspection of the time-frequency representation of the recordings, that no relevant signal component exceeded that frequency band. Afterwards, the upper root-mean-square envelope of the signal was computed [34,35] and subsequently smoothed by a local regression using the weighted linear least squares method and a 1st-degree polynomial model applied by means of a sliding window of 200 ms. Then, the footprint of the velocimetric profile, containing the detected PW, was identified as the instant t_1 at which the envelope reaches 5% of its baseline-to-peak amplitude. Finally, the PW transit time, from wrist to insonation site, was computed as $\Delta t = t_1 - t_e$, where t_e is the instant at which the cuff pressure rose above 2 mmHg i.e., the instant at which the compression of the hand really begins and therefore it is also the instant at which the PW is generated (Fig. 2). The vPWV was then calculated as the ratio of the travelled distance (Δx = wrist-probe distance) and the PW transit time: vPWV = Δx / Δt. Occasional odd vPWV values, attributed to the failure of the algorithm due to low signal-to-noise ratio of the Doppler signal, were automatically identified as the values beyond three times the Mean Absolute Deviation and then removed.

The Heart Rate (HR) was computed, from the instantaneous heart rate derived from the ECG signal, as the average over a 20-s interval prior to each pressure pulse delivery in order to include several respiratory cycles, as HR is modulated by respiration. The vessel cross-sectional perimeter was

measured both before (P_{start}) and after (P_{end}) the delivery of the series of 8 compressive stimuli, while the PVP was estimated only at the end of each series.

A preliminary assessment of the transient effect of PLR on vPWV was performed in order to define the time interval over which the response to the manoeuvre could be evaluated. All the vPWV measurements were expressed as percentage change relative to the respective baseline value (average of all values in BSL1 or BSL2) and aligned in time with respect to the moment of legs raising; then, the linear regression (robust least-squares fit, based on a bisquare weighting of the residuals, as provided by the Matlab function '*fit*') of the entire data set was used to model the trend and to select the time at which the PLR effect on vPWV fell below 5 % (arbitrarily set upon visual inspection): only data points preceding that time were used to compute the average vPWV value during PLR and the others were excluded from the subsequent analysis. The same time interval was used to assess the effect on HR. This procedure for the definition of the time interval based on experimental data was adopted because the time course of the PLR induced effect on vPWV was not known *a priori*.

2.6 Statistics

As a first step, a multivariate analysis of variance (2-way repeated measurements MANOVA) was performed taking into account the absolute values of the entire set of variables, in order to evaluate the effect of the 2 within-subject factors i.e., the manoeuvre (BSL vs. PLR) and its repetition (1 vs. 2), and eventually assess their interaction. Prior check of multivariate normality assumption was performed by multiple univariate Shapiro-Wilk tests. Then, in order to evaluate the two above mentioned factors on vPWV alone (i.e., from a univariate point of view), a 2-way repeated measurements ANOVA was performed. Finally, since the experimental design didn't allow to apply post-hoc (no between-subject factors), multiple paired T-tests were performed in order to compare, separately, BSL1 vs. PLR1, BSL2 vs. PLR2, BSL1 vs. BSL2 and PLR1 vs. PLR2, for each variable. Reliability of vPWV response to PLR was assessed comparing the two consecutive PLR-induced changes with respect to the averaged baseline value (i.e., ΔvPWV), specifically assessing the effect

of the manoeuvre irrespective of the alterations in the baseline values, by means of paired T-test, Spearman correlation coefficient and single-measurement, absolute-agreement, 2-way mixed-effects model Intraclass Correlation Coefficient (ICC) [36]. Finally, the intra-subject variability of the vPWV measurements acquired during BSL1, was quantified by the coefficient of variation (CoV = STD / mean * 100), averaged across all subjects, while their level of reliability was assessed by the multiple-measurements, absolute-agreement, 2-way mixed-effects model ICC.

All the values reported in the results section are expressed in terms of MEAN ± STD and the level of significance, was set at 0.05 for each statistical test, unless otherwise reported.

3 Results

Single measurements of vPWV in resting conditions (BSL1) exhibited little intra-subject variability, as expressed by the CoV = 7.7 ± 2.9 %, and a good level of reliability, as expressed by the ICC = 0.87 (95% confidence interval = 0.75-0.94).

In response to PLR vPWV transiently increased. The regression line fitted to the vPWV data, collected during PLR1 and normalized to baseline, exhibited a negative slope of 3.7 %/min and crossed the +5 % threshold at 179 s (~ 3 min), while during PLR2 the rate was 1.2 %/min and the cross happened at 242 s (~ 4 min).

On a multivariate basis (i.e., considering all the physiological variables measured) the 2-way repeated measurements MANOVA showed that PLR had an overall statistically significant effect ($p=0.02$) with no significant difference between PLR1 and PLR2 ($p=0.13$). However, on a univariate basis (i.e., considering only the vPWV absolute values) the 2-ways repeated measurements ANOVA showed that vPWV was significantly affected by PLR ($p<0.01$) and manoeuvre repetition ($p<0.05$). The results of the T-tests are reported graphically in Fig. 3, by means of symbols.

During PLR1 (Fig. 3) vPWV increased from 2.11 ± 0.46 to 2.30 ± 0.47 m/s ($p=0.01$), HR decreased slightly from 74 ± 7 to 70 ± 9 bpm ($p=0.02$), P_{start} increased from 17.9 ± 2.8 to 19.0 ± 3.4 mm ($p=0.04$)

while P_{end} was practically unaffected (p=0.44) and PVP increased slightly from 11.1 ± 1.9 to 11.6 ± 2.4 mmHg (p=0.05). The response to PLR2 (Fig. 3) was similar, with vPWV increasing from 1.93 ± 0.40 to 2.12 ± 0.46 m/s (p=0.01), while HR change was no longer significant (p=0.77) and P_{end} remained significantly above the pre-PLR2 value (p=0.02).

In terms of percentage change, vPWV exhibited a large increment compared to the other variables: vPWV showed a variation of 10 ± 14 % and 10 ± 15 %, HR of -3 ± 4 % and 1 ± 11 %, P_{start} of 6 ± 10 % and 8 ± 8 %, P_{end} of 2 ± 7 % and 6 ± 9 and PVP of 4 ± 7 % and 6 ± 9 %, for PLR1 and PLR2 respectively.

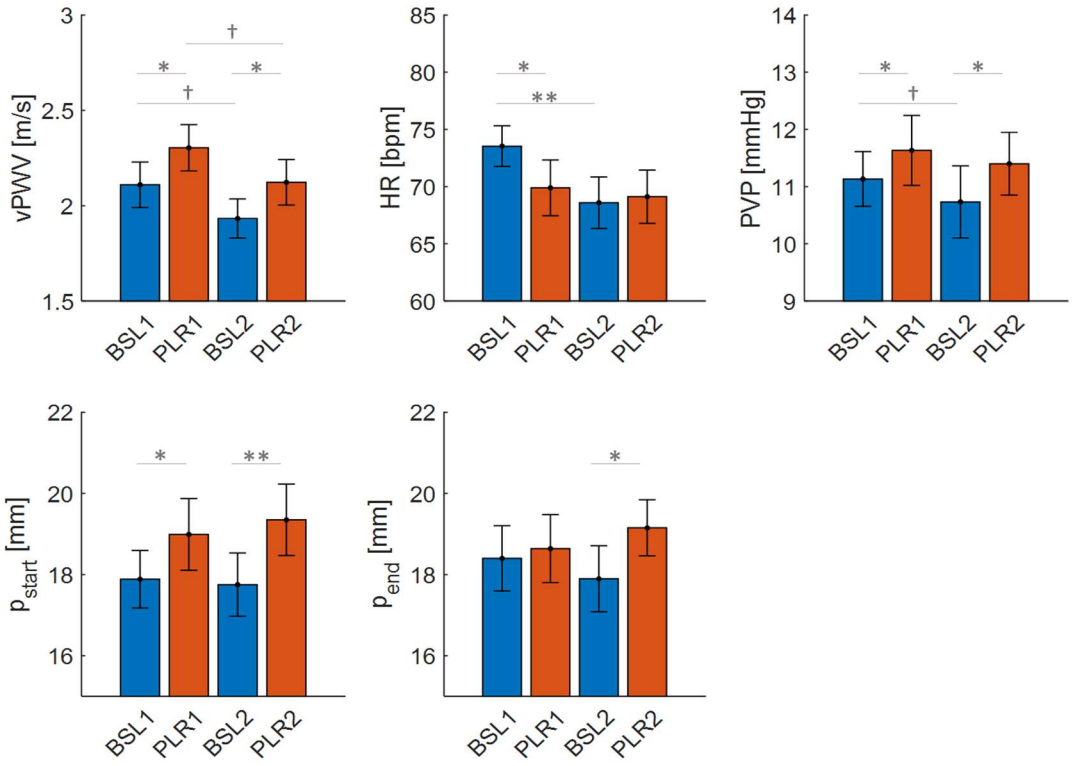

Fig. 3 *Average effect of PLR on physiological parameters. The blue bars represent the baselines values and the red ones the values recorded during PLRs. Error bars represent standard errors. Statistical significance, as assessed by paired T-test, is also reported (†: p<0.10; *: p<0.05; **: p<0.01)*

A comparison of the vPWV response to PLR1 (X-axis) and PLR2 (Y-axis) for the different subjects is qualitatively shown in Fig. 4. It can be observed that most subjects responded similarly to the two manoeuvres (segments oriented at about 45 deg), while only few, having a small magnitude of response, exhibited markedly different patterns. On an individual basis, vPWV exhibited a mean increase of at least 5%, with respect to the mean baseline value, in 10/15 and 8/15 subjects (i.e., responders) in response to PLR1 and PLR2, respectively. It is worth to notice that the 8 responders to PLR2 were also responders to PLR1. Although the responses of vPWV (ΔvPWV) to PLR1 and PLR2 were moderately correlated (Spearman correlation coefficient: 0.56, $p<0.05$) and their level of reliability was moderate (ICC = 0.57, 95% CI = -0.36-0.86), they showed good agreement when compared by paired T-test ($p=0.96$).

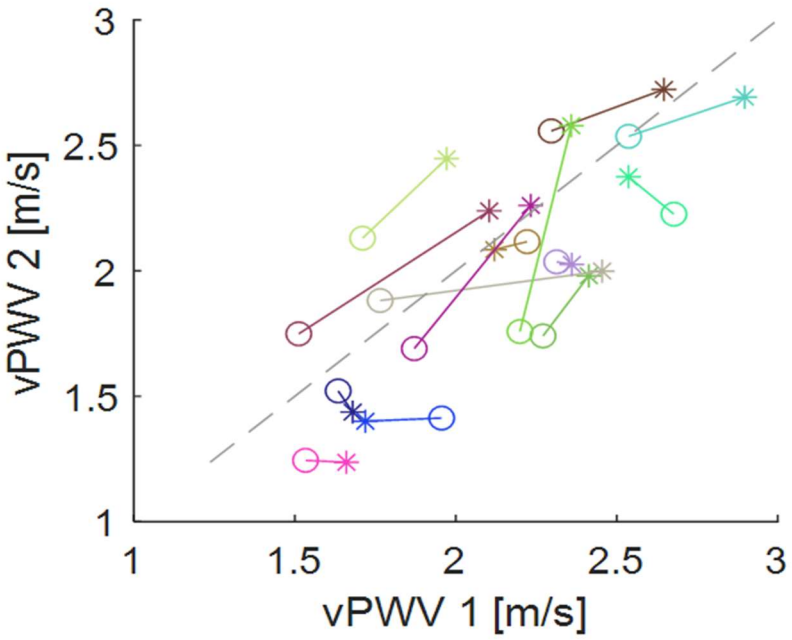

Fig. 4 *Comparison of vPWV responses to PLR1 and PLR2. The X-axis and Y-axis report the vPWV values during BSL1 and BSL2 (circles) and PLR1 and PLR2 (stars), respectively, straight lines joining circle and star of individual subjects. The dashed grey line is a 45-degree reference line indicating the slope corresponding to ideal reproducibility*

4 Discussion

With the present study, we showed for the first time that by assessing vPWV it is possible to detect simulated changes in blood volume, as produced by PLR in healthy subjects. Although transient in

nature (1-3 min) [5,26,37,38], the effect was quite consistently observed in 2 PLR manoeuvres performed in sequence.

In this respect, it is important to emphasize that the PLR from the supine position, as was performed in the present study, is a rather mild hemodynamic stimulus: indeed, in order to maximize the volume of blood that is displaced from the legs to the rest of the body, it is generally advisable to start the PLR from the semi-recumbent position, in which case the amount of displaced blood is estimated in the order of 300 ml [26,39]. Based on the observation that the cross-sectional area of the superficial femoral vein decreases by approximately 50 % when moving from the semi-recumbent (60-deg inclination of the trunk) to the supine position [25] we can roughly estimate that the blood volume displaced by the PLR is also reduced by the same amount, if starting from the supine rather than the semi-recumbent position, i.e., resulting in about 150 ml. For this reason, PLR from supine may be little effective [26,40] and result as a poor predictor of fluid responsiveness [12,41]. In the present study, we could not start from the semi-recumbent position, due to the methodological constraints related to the vPWV measurement in upper limbs. In spite of the relative weakness of the PLR-from-supine manoeuvre confirmed by the small changes observed in PVP, HR, and basilic vein size, vPWV effectively detected the hemodynamic challenge, exhibiting an overall significant increase and moderate repeatability in the response to the two manoeuvres. Notably, a PLR-induced increase in vPWV larger than 5 % was observed only in about 2/3 of the subjects, which can be ascribed to the weakness of the stimulus as well as to individual differences in basal volume status, in the compliance of central venous compartments and in autonomic reactivity. This result is in line with other studies which identified responders and non-responders to simulated fluid challenges [37,42].

To our knowledge, the vPWV variation in response to a real or simulated fluid challenge has not been previously investigated in humans. The only similar study was performed in anaesthetized dogs during progressive haemorrhage [24]. Interestingly the authors already noticed better sensitivity of vPWV to blood loss, as compared to standard haemodynamic parameters such as arterial blood pressure, highlighting the potentiality of this parameter, that, it is worth remembering, takes into

account not only the pressure alone but the working status of the vessels in terms of compliance, which is a more holistic approach. Unfortunately, after few investigations carried out in the seventies [20–24] the interest on vPWV decreased, possibly due to the lack of proper instrumentation and/or to the high variability in the measurement, e.g. CoV = 14% [23].

The low variability (CoV about 8 %), good reliability (ICC = 0.87) and, consequently, the good sensitivity of vPWV to simulated changes in blood volume achieved in the present study likely depends on the methodological arrangements implemented in the measurement. In particular, the generation of the compressive stimuli was synchronized both with respiration and with the ECG, which allowed to deliver the pulse always in the same respiratory phase (end of expiration) and in the same phase of the cardiac cycle. In this way, we could get rid of two major disturbing factors given that venous blood flow and pressure are affected by large respiratory modulation [22,23,43], as well as by cardiac perturbations, backward propagating from the right heart and/or directly transmitted from pulsating neighbouring arteries [22,43,44]. In addition, the implementation of a dedicated algorithm to automatize the footprint detection and therefore the vPWV estimation, allowed us to obtain a totally operator-free and objective measurement.

The vPWV variation in response to PLR1 was not correlated with that of PVP. This is only apparently in contrast with previous observations reporting a dependence of vPWV on venous pressure [20–23,25]. In fact, in the present case, each point refers to a different subject and the changes in venous pressure are in the order of 1 mm Hg or less, i.e., a very low value and, as such, poorly measured by the non-invasive technique adopted. However, PLR also produced a significant increase in BV size, as indicated by P_{start} (+ 6%). It should also be observed that a larger figure would be exhibited by a vessel size expressed in terms of cross-sectional area (which reflects the changes in volume, the vessel length being constant). On this basis, it may be reasonably concluded that the simulated increase in blood volume by PLR affected mainly the “unstressed volume” of the upper body [3,4].

It is interesting to analyse the post-effects of PLR by comparing BSL2 vs. BSL1. While the BV size is unchanged, there is a tendency towards lower PVP (p=0.08), and an almost significant decrease in

vPWV (p=0.06) (see Fig. 3): this is suggestive of a decreased sympathetically-mediated vascular tone of the venous compartment. The hypothesis of reduced sympathetic outflow is in line with the observed concomitant decrease in HR (Fig. 3) and find supports in the literature. In fact, it has been reported that central volume loading, as can be obtained for example by head-down tilt [45] or lower body positive pressure, produces sympathetic inhibition [46], with effects that may outlast the duration of the stimulus [47]. It is remarkable that, in spite of the post-effects of PLR1 and the ensuing differences between BSL1 and BSL2, the vPWV response to PLR2 was still quite well correlated to PLR1 (Fig. 4), achieving moderate reliability.

4.1 Potential for clinical applications

Besides a few potential drawbacks, namely the complexity of equipment and experimental set-up and the necessity to operate on a full limb, the proposed technique has several appealing characteristics for clinical applications. First of all, this measurement has a high sensitivity to mild hemodynamic challenges, whose demonstration is a major outcome of the present study. Secondly, the measurement is objective, the intervention of the operator being limited to positioning electrodes and probes and in selecting the delay for appropriate delivery of the pulse with respect to the R-wave of the ECG. Thirdly, the measurement is non-invasive and can be repeatedly performed. The maximum time resolution (related to the maximum frequency of the measurement) has not been specifically tested, as yet. A minimum time interval is required for the limb extremity to refill and this may depend on the actual circulatory conditions. A frequency of about 2-3/min as operated in the present study is rather high and adequate to describe fast hemodynamic transients, such as the response to PLR.

With these characteristics the technique is adequate for long term patient monitoring and, for example, it could be potentially useful to provide quantitative and objective monitoring of progressive vascular filling during fluid administration in depleted patients or for early detection of excessive fluid depletion in patients undergoing dialysis. As for the capacity of vPWV to predict fluid

responsiveness, additional studies are necessary in which vPWV is compared to other parameters, like cardiac output or stroke volume, in response to simulated fluid challenges.

5 Limitations

The subjects had to actively signal the end-expiratory phase. In future studies, the automatic detection of the end-expiratory phase should be implemented in order to avoid any active involvement of the subject, which could possibly influence the autonomic balance and affect the measurement.

Due to the above limitation, the measurements were not perfectly timed with respect to the start of PLR, in addition, the different variables were measured at different times. This may have underestimated the maximum variations exhibited by the different variables. In fact, it is generally known that adaptation occurs in the system and that the hemodynamic effect of PLR tends to fade away within minutes. This phenomenon appears poorly described in the literature [37,48] but it deserves attention as it could reveal additional characteristics of the body response to fluid challenges. In this respect, vPWV could be one of the meaningful variables to consider.

Finally, the comparison between PLR1 and PLR2 cannot constitute a real repeatability study, since the two manoeuvres were separated by a short resting interval and the variables were not completely returned to control (pre-PLR1) levels.

6 Conclusions

The vPWV was shown to effectively detect a mild central volume loading, as obtained by the passive leg raising form the supine position, which produced only minor changes in peripheral venous pressure (<1 mmHg). This new hemodynamic index is objectively and non-invasively assessed, sensitive to mild hemodynamic challenges and characterized by low variability and good reliability of the measurement. For these reasons, it appears to have great potential for clinical applications.

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Declaration of Interest

The authors LE, CDB, CF, and SR have submitted a patent application concerning the assessment of venous pulse wave velocity.

Authors' contribution

LE: Conceptualization, Methodology, Software, Formal Analysis, Data Curation, Writing-Original draft, Writing - Review & Editing, Visualization. **NEC:** Software, Validation, Formal Analysis, Writing-Original draft, Visualization. **CDB:** Methodology, Writing - Review & Editing. **CF:** Methodology, Resources, Writing - Review & Editing, Supervision. **SR**: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision.

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