Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–13, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2016.01.010

● *Original Contribution*

PITFALLS OF DOPPLER MEASUREMENTS FOR ARTERIAL BLOOD FLOW QUANTIFICATION IN SMALL ANIMAL RESEARCH: A STUDY BASED ON VIRTUAL ULTRASOUND IMAGING

ABIGAIL SWILLENS,* DARYA SHCHERBAKOVA,* BRAM TRACHET,*† and PATRICK SEGERS*

*IbiTech-bioMMeda, Department of Electronics and Information Systems, Ghent University, Ghent, Belgium; and †Institute of Bioengineering, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

(*Received* 18 *June* 2015; *revised* 14 *January* 2016; *in final form* 19 *January* 2016)

Abstract—High-resolution Doppler is a popular tool for evaluating cardiovascular physiology in mutant mice, though its 1-D nature and spectral broadening processes complicate interpretation of the measurement. Hence, it is crucial for pre-clinical researchers to know how error sources in Doppler assessments reveal themselves in the murine arterial system. Therefore, we performed virtual Doppler experiments in a computer model of an aneurysmatic murine aorta with full control of the imaging and insonified fluid dynamics. We observed significant variability in Doppler performance and derived vascular indices depending on the interrogated flow, operator settings and signal processing. In particular, we found that (i) Doppler spectra in the upper aortic branches and celiac artery exhibited more broadening because of complex out-of-beam flow paths; (ii) mean frequency tracking outperforms tracking of the outer envelope, but is sensitive to errors in angle correction; and (iii) imaging depths deviating much from the elevation focus suffer from decreased spectral quality. (E-mail: abigail.swillens@ugent.be) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Pulsed Doppler, Small animal imaging, Ultrasound simulation.**

INTRODUCTION

The advent of genetically engineered mice that model human cardiovascular pathologies has initiated a still ongoing quest for new and better non-invasive monitoring techniques to evaluate the pathophysiologic changes occurring in these animals and for phenotyping. Typically, imaging methods have been adapted from the human setting, but the small size and high heart rates in mice place extreme demands on both spatial and temporal resolution (Hartley et al. 2002), challenging appropriate system development. Because of their ease of use, ultrasonic imaging techniques have been purposefully modified to meet these high-resolution requirements. In particular, high-resolution Doppler systems have become a popular tool for evaluating cardiovascular physiology in mutant mice.

Previous Doppler investigations in mice have convincingly indicated that the shape of the temporal blood velocity signals in the heart and major blood vessels can indeed be related to pathologic changes in cardiovascular functioning. Analysis of the timing and height of the peaks in cardiac Doppler signals may reveal left ventricular systolic and diastolic (dys)function, as reported by Taffet et al. (1996) in a hyperthyroid and senescent mouse model. In an atherosclerotic mouse model, an anomalistic waveform was detected in the aortic arch, indicating a biphasic acceleration phase, potentially caused by the increased wave reflections resulting from the stenosed carotid bifurcation in these animals (Hartley et al. 2000; Li et al. 2003). In a mouse model of cardiac hypertrophy (transverse aortic constriction), Li and co-workers found the peaks in the carotid velocity waveforms at the time of the intervention to be correlated with the degree of hypertrophy 2 wk afterward (Hartley et al. 2002; Li et al. 2003). Further, Doppler spectrogram studies in mice have been reported at the level of the renal artery (Bonnin et al. 2008; Sullivan et al. 2009), hepatic artery (Bonnin et al. 2007), coronary artery (Saraste et al. 2001) and umbilical artery (Mai et al. 2004).

In addition to this cardiovascular phenotyping based on flow waveform analysis, Doppler signals can also be

Address correspondence to: Abigail Swillens, IBiTech-bioMMeda Research Group, UZ Campus Heymans, Blok B (5th floor), De Pintelaan 185, 9000 Ghent, Belgium. E-mail: abigail.swillens@ugent.be

used for more in-depth characterization of hemodynamics or tissue mechanics. As such, Doppler data can be combined with simultaneous pressure or diameter measurements to reveal pathologic changes in vascular impedance, that is, the opposition to pulsatile blood flow in an artery (indicative of peripheral perfusion), as reported in an atherosclerotic mouse model by Hartley et al. (2000). Further, in the same apoE-knockout mouse model, wave intensity was found to be a powerful parameter with which to analyze the nature of arterial wave reflections in the carotid artery (Reddy et al. 2009). Combining Doppler info from two measurement sites allows reliable determination of the arterial pulse wave velocity (an index of arterial stiffness), as reported by Hartley et al. (1997), Williams et al. (2007) and Trachet et al. (2015). Finally, Doppler velocities can also be used as a substitute or support for local blood pressure measurements. Indeed, Doppler velocities allow estimation of the pressure drop over a stenotic region *via* the simplified Bernoulli equation ($\Delta P = 4V^2$) (Hartley et al. 2002; Li et al. 2003), as described in the cardiac hypertrophy mouse model (Li et al. 2003). A Doppler flow cuff has been developed to assess absolute systolic–diastolic pressure measurements in the mouse tail (Reddy et al. 2003).

Recently, a new application of pulsed Doppler techniques has emerged as computational fluid dynamics is increasingly used to gain insight into animal hemodynamics (flow velocities and pressures) and derived parameters (*e.g.,* wall shear stress, vorticity). Indeed, numerical techniques can overcome the shortcomings of current clinical imaging modalities such as ultrasound and magnetic resonance imaging, but require *a priori* knowledge of the hemodynamic conditions at the boundaries of the simulated flow domain, often provided by *in vivo* Doppler measurements as described in Trachet et al. (2011).

Although Doppler techniques are considered an established method for flow screening, concerns have arisen regarding their accuracy. In particular, the inherent 1-D nature of the measurement and the spectral broadening processes (Evans and McDicken 2000) complicate appropriate processing and interpretation of the acquired Doppler spectra and waveforms. Indeed, with respect to the first limitation, Doppler velocities capture the blood flow component only in the direction of the ultrasound beam; thus, an estimate of the true flow direction is required to obtain reliable quantitative velocity data ("angle correction"). The second complicating factor hampers univocal determination of the true velocity in the interrogated sample volume, as a full spectrum of Doppler frequencies is picked up, even in the theoretical case of one insonified red blood cell.

Although recent research has come up with innovative solutions to overcome the 1-D limitation and to improve the reliability of analyzing Doppler spectra (cf. Discussion), current commercial scanners for small animal research are still equipped with the traditional Doppler techniques. This can probably be attributed to the fact that the recent technologic advances necessitate increased system demands and still lack proven robustness in physiologically relevant settings. Furthermore, over the past decades, most clinical research in human adults has been performed with regular pulsed Doppler, which would justify its use in mice to allow the link with the human setting.

Hence, it is necessary for pre-clinical researchers to be aware of the confounding error sources in pulsed Doppler assessments and how they might reveal themselves in the murine arterial system. For this purpose, we performed virtual Doppler experiments in a computer model of a murine aorta with a pharmacologically induced ascending and abdominal aortic aneurysm. Our applied simulation tool was previously found to be useful in analyzing the performance of new and existing ultrasonic blood flow estimators in the human carotid artery (Swillens et al. 2010b, 2010c), as it allows comparison of the virtual ultrasound data with the true hemodynamics present behind the image, even under the most complex flow conditions. As such, in the Methods section of this article, the different steps involved in the simulation environment are disclosed, and in the Results section, the different Doppler spectra obtained in seven aortic side branches, as well as the ascending and distal abdominal aorta, are presented. Quantitative analysis of these Doppler data is performed by comparing the original and angle-corrected velocities, as well as derived vascular indices (*e.g.,* pulsatility index), with the ground truth data. Finally, in the Discussion, we give a more in-depth analysis of why the different measurement locations may result in highly different Doppler performance.

METHODS

A previously developed multiphysics modeling approach (Swillens et al. 2009, 2010a) was applied to perform virtual Doppler experiments in a mouse-specific aorta and its branches. The key component of this approach is an ultrasound simulator, which models the blood flow as an ensemble of point scatterers reflecting the ultrasonic waves emitted by the modeled probe. During the simulated scan, the point scatterers are moved according to realistic blood flow patterns to enhance the realism of the synthetic ultrasound data. For this purpose, the movement of the scatterers is derived from velocity vector fields as obtained from computational fluid