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Short Communication

Reference intervals for whole blood viscosity using the analytical performance-evaluated scanning capillary tube viscometer

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ABSTRACT

Objectives: This study was performed to establish the reference intervals for whole blood viscosity (WBV) using the analytical performance-evaluated scanning capillary tube viscometer (SCTV).

Design and methods: The analytical performance of the SCTV was evaluated using three different levels of QC materials and sixty human EDTA-blood samples. To establish the reference intervals for WBV, 297 healthy individuals (123 men and 174 women) were selected from 1083 subjects.

Results: Within-day precisions with QC materials and human whole blood and between-day precisions with QC materials were below 5.0%, 6.6% and 8.0% in CVs at all shear rates, respectively. Comparison tests between the SCTV and the Brookfield viscometer showed a significant correlation ($R^2 = 0.972$, $p < 0.001$). The reference intervals for WBV in healthy men were 3.66–5.41 cP at 300 s^{-1} and 23.15–36.45 cP at 1 s^{-1} while those in women were 3.27–4.32 cP at 300 s^{-1} and 18.20–27.36 cP at 1 s^{-1}, respectively.

Conclusions: Using the analytical performance-evaluated SCTV, the reference intervals for WBV were established in healthy adults, which could be beneficial to the clinical utility of WBV in the aspect of appropriate modalities for the improvement of blood viscosity.

Introduction

Whole blood viscosity (WBV) indicates the inherent resistance of blood flow. WBV is one of the most crucial hemodynamic parameters which can determine the flow regime of whole blood in vessels, such as the shear stress at vessel walls and the oxygen delivery to tissues [1]. The WBV has been reported to be independently correlated with the well-known cardiovascular risk factors, such as hyperlipidemia, diabetes, hypertension, obesity, cigarette smoking, male gender, and aging [2,3]. In addition, epidemiologic studies on the relationship between WBV and the occurrence of major cardiovascular events (i.e., cardiovascular death, acute myocardial infarction, or acute need for cardiovascular surgery) have revealed that the risk of such events increases with an elevated WBV, representing a potential application of WBV as a biomarker to predict the risk and severity of ischemic cardiovascular diseases in conjunction with conventional cardiac risk factors [4].

Whole blood exhibits a non-Newtonian behavior of which viscosity decreases according to the increasing shear rates as a result of complicated interactions between the cells and molecules. The studies to evaluate these characteristics of WBV have been established, but are mostly based on rotational viscometers such as coaxial cylinder (i.e., Contraves viscometer) and cone-and-plate (i.e., Brookfield viscometer) types [5]. However, both viscometers have not been practically used in the clinical laboratory because they require manual cleaning between tests and thus contain a potential risk of contamination by direct contact with blood. In addition, since the mechanism of a rotational viscometer is based on the torque measurement at a single predefined rotational speed (or shear rate), it has been practically limited in fully describing the complex behavior of WBV over a physiologic range of shear rates, i.e., from 1 to 1000 s^{-1} [6]. Also note that because both Contraves and Brookfield viscometers were designed to utilize a sensitive spring to measure wall shear stress, the accuracy of wall shear stress measurements critically depends on the correct calibration of the spring, which requires a periodic calibration of the spring at a factory [5].

Recently, a new scanning capillary tube viscometer (SCTV) to overcome the drawbacks of conventional rotational viscometers has been introduced, which determines the WBV profiles over a range of shear rates from 1 to 1000 s^{-1} with a single automated measurement. Using

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a gravity-driven disposable U-tube system, the SCTV (BVD-PRO1, BioVisco Inc., South Korea) does not require both manual cleaning between tests and periodic calibration at factory [6–8]. In order to eliminate the effect of temperature variation on viscosity, both blood samples and a disposable U-tube were preheated and maintained to 36.5 ± 0.5 °C during viscosity measurement using a built-in electronic heating system in the SCTV.

Thus, the potential utility of the SCTV to measure WBV has been highlighted in clinical practice. However, the establishment of appropriate reference intervals for WBV over a range of shear rates from 1 to 1000 s^{-1} is essential to interpret a set of results for a cardiovascular patient and determine a proper therapeutic treatment. Therefore, the objective of this study was to establish the reference intervals for WBV in healthy men and women over a range of shear rates from 1 to 1000 s^{-1} after conducting the analytical performance evaluation of the SCTV through precision and comparison tests for a convincing demonstration.

Materials and methods

Analytical performance evaluation of the SCTV

The analytical performance validation of the SCTV was conducted according to the guideline (EP-5A2) of the Clinical Laboratory and Standards Institute (CLSI) [6,9]. Within-day precision tests of the SCTV were carried out with three different quality control (QC) materials (i.e., high, intermediate, and low levels of viscosity) for twenty separate viscosity measurements (sixty measurements in total) and with human whole blood collected from each of six different individuals for ten separate viscosity measurements (sixty measurements in total). Between-day precision tests were performed with three different QC materials over twenty consecutive days, where four viscosity measurements a day with two in the morning and another two in the afternoon were made for each QC material.

In this study, prior to the process of establishing the reference intervals for WBV, analytical performance of the SCTV for WBV measurement was carefully evaluated to ensure convincing reference intervals using the QC materials with three different concentrations of 23.7, 26.9, and 32.0 wt.% of a maltose solution for low-, medium-, and high-viscosity ranges, respectively. Here, the ranges of low, medium, and high viscosity were given as approximately 9, 22, and 56 cP at a shear rate of 1 s^{-1}, and 3, 4, and 7 cP at a shear rate of 300 s^{-1}, respectively [6]. As a result of both repeatability and long-term stability tests for the QC materials, they could mimic the non-Newtonian shear-thinning behavior of whole blood having coefficients of variations less than 5% [10].

For comparison tests, WBVs were measured at a high shear rate of 300 s^{-1} with 133 whole blood samples using the SCTV and a conventional rotating Brookfield viscometer (LV-III with CP-40, Brookfield Engineering, MA). Here, the comparison test at a low shear rate could not be performed because the latter viscometer was limited to precisely measure the WBV at high shear rates. During systole, when blood flows at a relatively high velocity over 30 cm/s in large arteries (i.e., coronary and carotid), such a high-shear-rate condition (i.e., 300 s^{-1}) could occur, and WBV becomes mostly governed by the inertia force. On the contrary, when blood moves slowly at a flow velocity less than 0.01 cm/s during diastole, a corresponding low-shear-rate condition (i.e., 1 s^{-1}) could occur with WBV mostly governed by the viscous force [6].

Study subjects and laboratory analyses

The reference intervals for WBV were determined according to the guidelines of CLSI and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [11–13]. Subjects who participated in the present study were recruited from those who visited the Health Promotion Center at Chonbuk National University Hospital, South Korea. The study protocol was approved by the institutional review board for medical ethics. The total number of subjects who filled out questionnaires and gave written consents for participation was 1083 (633 men and 450 women). Complete blood count, blood chemistry tests, and immunoassay for 1083 subjects were carried out using Sysmex XE-2100 (Sysmex, Kobe, Japan), ADVIA 2400 chemistry system, and ADVIA Centaur XP immunoassay system (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY), respectively. Blood samples were drawn in the morning after fasting for a minimum of 12 h.

Among the 1083 recruited subjects, based on the written questionnaires and the results of blood tests, those who were excluded in the following criteria were chosen as healthy subjects; any diseases currently under therapy and medication use, diagnosed hypertension, blood pressure over 140/90 mm Hg at examination, diagnosed obesity, current/recent hospitalization, fasting blood glucose over 126 mg/dL, hemoglobin concentration below 12 g/dL (male) and 11 g/dL (female), blood platelet count below normal, cigarette smoker, oral contraceptive user, pregnancy, blood donor, history of transfusion, recent alcohol consumption (over 2 times a week), any abnormalities in blood chemistry tests, HBsAg positive, anti-HCV Ab positive, and past history of major surgery (thyroid, uterus, ovary, etc.).

WBVs of the selected healthy subjects were then measured to determine the reference intervals for WBV using the SCTV at 36.5 ± 0.5 °C.

Statistical analyses

The normal distribution of the obtained WBV data was analyzed and outliers were excluded with a D/R ratio method. The nonparametric method was used to calculate the 2.5 and 97.5 percentile reference limits when the data did not fit a normal Gaussian distribution, as recommended by CLSI and IFCC [11]. The reference intervals for WBV were separated for two different subclasses, males and females. Comparisons between the two groups were made and all continuous variables were described as mean ± standard deviation (SD). All analyses were 2-tailed, with a clinical significance defined as a P value < 0.05. Statistical processing was carried out using SPSS-PC Version 18.0 (SPSS-PC Inc., Chicago, IL, USA).

Results and discussion

As shown in Table 1, within-day precisions for WBV measured by the SCTV with three different QC materials and human whole blood were within 5% and 6.6% in the coefficients of variation (CVs), respectively. Similarly, between-day precisions of WBV measurements conducted over twenty consecutive days were below 8% in CV, resulting in good analytical performance over shear rate ranges from 1 to 1000 s^{-1}. In comparison to tests at a shear rate of 300 s^{-1}, an interchangeable correlation was found between the SCTV and the Brookfield viscometer with a linear regression model $y = 0.995x + 1.0318$ ($R^2 = 0.972$, $P < 0.001$).

The rheological feature of whole blood can be described using both yield stress (a minimum shear stress to maintain a continuous blood flow) and shear-thinning behavior (a decreasing viscosity with the increase of shear rates). Among several constitutive models for non-Newtonian fluids, the Casson model has been considered to be the most accurate in addressing these rheological properties of blood flow [14]. Thus, the shear-thinning non-Newtonian flow characteristics of whole blood was calculated using the Casson model in which a yield stress term was included:

$$\begin{array}{ll} \sqrt{\tau} = \sqrt{\tau_y} + \sqrt{k}\sqrt{\dot{\gamma}} & \text{when } \tau \geq \tau_y \\ \dot{\gamma} = 0 & \text{when } \tau \leq \tau_y \end{array} \quad (1)$$

where τ denotes the shear stress by blood flow, τ_y is the yield stress, k is a Casson model constant, and $\dot{\gamma}$ is the shear rate (the rate of shearing deformation). Since viscosity η is mathematically defined as the ratio