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Experimental study of pulsatile blood flow in micro channels

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Abstract

The study of the blood rheology and of the dynamical characteristics of its flow is a very important step towards comprehension, prediction, diagnosis and therapy of many cardiovascular diseases. Blood is a complex multiphase system which in general exhibits a non-Newtonian behavior. Since, due to coagulation, conducting experiments with blood is a difficult task, blood mimicking fluids, i.e., fluids with similar rheological properties, are used. In this study an aqueous glycerin solution containing small amounts of xanthan gum, which can be considered a successful blood analogue is employed, while a solution of glycerin is used as reference Newtonian fluid. The experiments are conducted in a 600μm hydraulic diameter micro channel (matching arteriole dimensions) comprising a bifurcation. With the aim of reproducing physiological flow conditions pulsatile flow is generated using a syringe pumps. The local velocities in the micro channel were measured using micro Particle Image Velocimetry (*μ-PIV*), a non-intrusive technique. Consequently, the velocity distribution and the wall shear stress as well as their variation during the period between two consecutive pulses have been calculated. It was found that the assumption that blood behaves as Newtonian fluid does not hold true in arterioles and for low *Reynolds* numbers. The use of a Newtonian fluid leads to overestimated wall shear stresses and this difference ranges from 30 to 40%. Finally it was verified that the outer wall of the bifurcation due to lower shear stresses, is more predisposed to plaque formation as in large arteries.

Keywords: *Non-Newtonian fluid, pulsatile flow, μ-PIV, arterioles, wall shear stress, atherosclerosis*

Introduction

The study of blood velocity distribution in blood vessels can be proved very important for medicine and biomedical engineering. Moreover, blood flow modeling is helpful in therapy strategy; it can help to make the choice between surgical and interventional procedures. Biomechanical studies can also be aimed at designing surgical repairements and implantable medical devices and predicting effects of implanted prostheses and devices (Thiriet, 2007). Recent papers (e.g. Chatzizisis and Giannoglou, 2006; Shaaban and Duerinckx, 2000) report that local hemodynamic forces affect the generation and the progression rate of atherosclerosis. The aforementioned studies report that the atherosclerotic plaques occur mainly in regions of curvature, bifurcation, and branching of the vessels. A low wall shear stress promotes the formation of atherosclerotic plaque (Chatzizisis and Giannoglou, 2006), while, on the other hand, high shear stresses can cause the release of nitric oxide and prostacyclin which act as vasodilators and can protect the endothelial cells (John, 2009).

Figure 1a (Chatzizisis and Giannoglou, 2006) describes the effect of wall shear stress on the formation and progression of atherosclerotic plaque. First the low blood velocity, i.e. low wall shear stress, leads to an increased concentration of particles responsible for atherosclerosis, i.e low density lipoprotein, while at the same time causes a raise to endothelium layer permeability. These phenomena enhance the subendothelial migration of blood atherogenic particles through endothelium, promoting lesions forming (***Figure 2***). The further progression

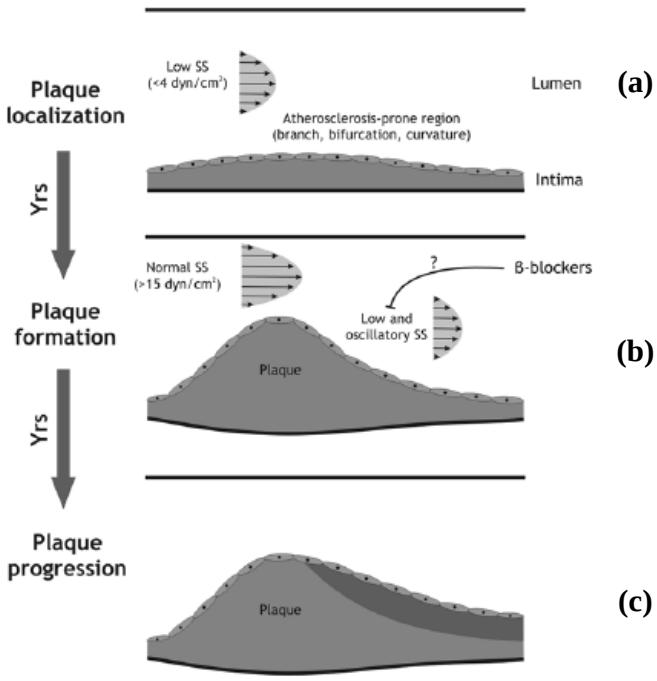

Figure 1: Plaque formation and progression due to low shear rates (Chatzizisis and Giannoglou, 2006).

of the lesion formation (***Figure 1b***) leads to an artery stenosis, which acts as a backward-facing step. It is known (e.g. Mouza et al., 2005) that the minimum wall shear stress on a stepped wall occurs in the neighborhood of the reattaching flow region, that is just behind the backward-facing step. This leads to a further development of the plaque (***Figure 1c***). Considering all the aforementioned phenomena it is obvious that biomechanical factors are significant in atherosclerosis and their study can be proved useful in predicting and treating the disease at an early stage.

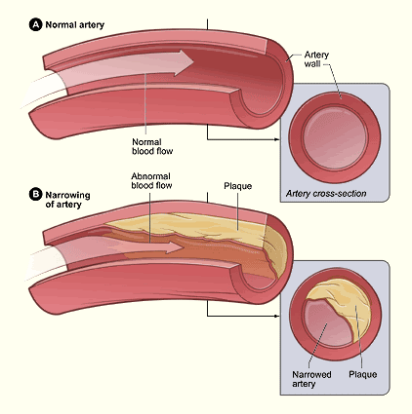

Figure 2: Lesion in an artery (A.D.A.M. Medical encyclopedia).

Numerous works have been published concerning atherosclerosis and wall shear stress for large arteries and specifically the coronary artery (e.g. Artoli et al., 2006; Giannoglou et al., 2006; Gijsen et al., 1999a; Gijsen et al., 1999b; Huo and Kassab, 2006; Long et al., 2000). On the contrary few researchers have studied the flow in small blood vessels (Riva et al., 1985).

According to the Newton's law of viscosity a fluid is considered Newtonian when its viscosity is constant and independent of the shear stress. On the other hand fluids whose shear stress vs. shear rate relationship does not follow this law are known as non-Newtonian. Such a fluid is blood and its viscoelastic properties rely on its chemical composition (Fournier, 2007). Blood is a multiphase mixture of plasma, a Newtonian fluid, and three main cell types, red blood cells, platelets and leukocytes. Under low shear stress conditions normal red cells in plasma form linear aggregates (rouleaux) which disrupt flow streamlines and greatly increase whole apparent blood viscosity. By increasing the shear rates these aggregates are progressively deformed and consequently the apparent viscosity decreases to the asymptotic limit of 3.5 cP (Fournier, 2007). The viscoelastic properties of blood greatly affect its flow dynamics (Gijsen et al., 1999a). Many researchers made the assumption that blood can be considered a Newtonian fluid, due to the high shear rates existing in large arteries (e.g., Long et al., 2000, Mabotuwana et al., 2007, Stamatopoulos et al., 2010) but Gijsen et al. (1999a) proved that this is not accurate enough as the viscoelastic properties affect the velocity distribution.

It is known that blood flow *in vivo* is unsteady. The pressure and the volumetric flow rate of the blood vary with time over the period of heart relaxation and contraction. Blood flow is divided into two phases: *systole*, during which heart is pumping the blood, and *diastole*, during which heart is pumping no blood and ventricles are filled up. The pressure and velocity pulse change with distance from the heart, the maximum pressure declines and the pulse width broadens with increasing distance. In arterioles the pressure change is greatly attenuated in contrast with some vessels (e.g. the external carotid artery and the femoral artery), where there is a period of negative velocity (Truskey et al., 2004).

It is also known that *in vivo* the walls of blood vessels are not rigid. The effect of arterial wall elasticity on the flow is difficult to be studied experimentally, because materials that match the elastic behavior of arteries accurately are still not available. Although in large arteries there is a significant change in vessel diameter during the pulse, in small vessels i.e. arterioles, where the pressure change is greatly attenuated, it can be assumed that the change in diameter is not significant and thus the walls can be considered rigid.

The ***scope*** of this study is to measure the velocity distribution of a blood analogue fluid (since the use of real blood is problematic mainly due to coagulation and lack of optical transparency) in a bifurcation which is considered a high risk geometry. Apart from the non-Newtonian blood analogue, a Newtonian fluid will be also employed with the intention to study the way in which the different behavior of the fluid affects the wall shear stress during pulsatile flow for relatively low *Reynolds* numbers. The wall shear stress as well as its variation between two consecutive pulses will be calculated from the velocity data.

Velocity measurement methods

Measuring velocity *in vivo* is something difficult to achieve. In most works experiments are conducted *in vitro,* using methods like Laser Doppler Anemometry (Gijsen et al., 1999a, Gijsen et al., 1999b) and Ultrasonic Velocimetry (Siouffi et al., 1984). These techniques can be used to measure two, and even three components of velocity.

A relatively new method for velocity measurements in micro channels is micro Particle Image Velocimetry (*μ-PIV*), which is a non-intrusive technique for measuring two dimensional velocity fields. There are several works that use *μ-PIV* to study biological systems (Jung Yeop Lee, 2007; Sugii et al., 2002; Vennemann et al., 2006). The flow tracing particles are seeded in the fluid. The choice of these particles is crucial for the validity of

the measurements. They must be small enough to accurately follow the fluid motion and to avoid micro channel obstruction and in the same time large enough to be adequately imaged and to avoid effects like Brownian motion. In general, two successive images of the particles on the plane of interest are required, that corresponds to a known and sort time interval (t). After pre-processing, background removal and signal intensification, the cross correlation of the two images provides the displacement (Δs) of the particles on the focusing plane. For the known t and the measured Δs, the 2-D velocity U on this plane can be calculated from the following equation.

$$\boldsymbol{U=\Delta s/t} \qquad (1)$$

The most important constriction to the use of μ-*PIV* is that the vessel walls must be transparent, a fact that make *in vivo* measurements non feasible.

Wall Shear Stress Estimation

The use of special electro-diffusion probes mounted in the channel wall allows direct measurements of wall shear stress in the macro scale (e.g. Pantzali et al., 2008). However, the same measurements in micro-channels (i.e., arterioles) or in channels with elastic walls (i.e., large arteries) are not an easy task. It is common practice in these cases that the estimation of the wall shear stress can be accomplished by methods based on curve fitting of the measured velocity data. Lou et al. (1993) described several methods for wall shear stress estimation on arterial walls. According to them a good estimation requires that:

- the velocity is measured close to the wall,
- the method for the velocity measurement is non invasive, i.e., it does not disrupt the flow.

For the wall shear rate calculation an estimation of the slope of a velocity profile must be acquired from at least two, preferably three, velocity values obtained near the vessel wall. The accuracy of wall shear stress depends both on the spatial resolution of the measured velocity and the assumptions for the velocity profile near the wall. The simplest interpolation, called the ***linear*** method, assumes a linear velocity distribution and utilizes the velocity component in a single point away from the wall. If $\boldsymbol{u}$ is the component of velocity parallel to the wall measured at a distance $\boldsymbol{y}$ from the wall, then the wall shear rate can be approximated by the following equation:

Shear Rate = u/y (2)

A second method, called the *quadratic* method, assumes a quadratic velocity distribution between the velocity measurements at two points, located at different distances close to the wall. In another method, a third velocity is measured at a point between the two points used in the quadratic method. A least-squares fit method is adopted to accommodate all three points, or four points if the point at the wall is included (no slip condition), in a parabolic velocity profile. The benefit of the extra point is doubtful because it forces the velocity profiles to comply more with the data in the region farther away from the wall than with the data closer to the wall. Moreover, a higher order polynomial is generally less robust because the result becomes sensitive to small amplitude as the order of the curve fitting increases (Lou et al., 1993).

In the present work it is assumed that the velocity distribution near the wall is ***linear***. With the use of two measurements near the wall and a third which is the zero velocity on the wall surface, a fitting line is estimated (***Figure 3***). The ***slope*** of this line represents the shear rate near the wall. Substituting this value to the corresponding equation of viscosity, which depends on the fluid employed, a good estimation for the wall shear stress can be made. The main assumptions of this method are:

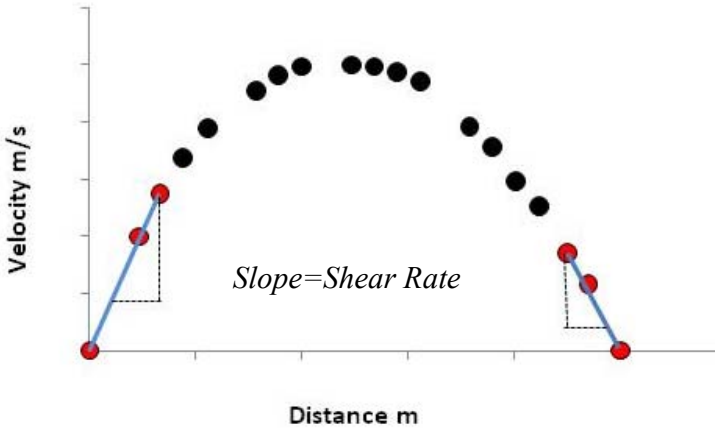

Figure 3: Estimation of wall shear rate from velocity data.

- The velocity is measured close to the wall.
- The velocity distribution near the wall is linear.

Experimental procedure and setup

With the intention to simulate the non Newtonian behavior of human blood an analogue was used. The analogue comprises 75% w/w distilled water, 25% w/w glycerin and xanthan gum, which is a polysaccharide used as food additive and as rheology modifier and

it is the cause of the non-Newtonian behavior of the fluid. The viscosity of this solution was measured by a cone plate reometer (*G1253-1006, Sigma-Alorich*). In ***Figure 4*** the viscosity of the blood analogue is compared with the viscosity of real blood given by Cho & Kinsey (1991) and Shin & Keum (2002). For shear rates ranging from 1 to 1000s^{-1} the viscosity of the blood analogue employed is in very good agreement with blood viscosity. The shear stress vs. shear rate relationship for the blood analogue follows the model by ***Herschel-Bulkley*** (***Eq***. ***3***) and is used for estimating the wall shear stresses. As a reference to the Newtonian behavior an aqueous solution of glycerin, with viscosity identical to the asymptotic viscosity of blood (3.5cP), was employed (62% w/w distilled water and 38% w/w glycerin).

$$Shear\ Stress = \boldsymbol{A} + \boldsymbol{B}(Shear\ Rate)^{\boldsymbol{C}} \qquad (3)$$

where A: yield stress 0.02160 (Pa)

B: viscosity coefficient 0.01345 (Pa $s^{1/c}$)

C: rate index 0.7812

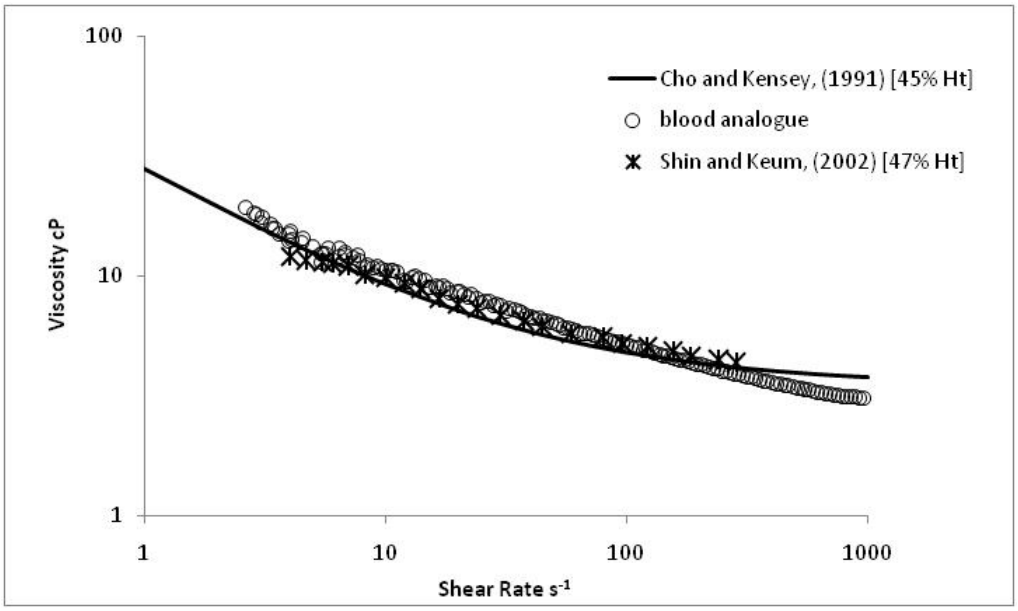

Figure 4: Comparison of viscosity between real blood and the blood analogue.

Aiming to mimic physiological flow conditions, pulsatile flow is generated using a syringe-pump (*Alladdin, Al-2000*). Assuming that flow rates in arterioles depend on the distance from the heart, two different pulses were used. As it is shown in ***Figure 5***, the first

one corresponds to regions with high flow rates (*case 1*: 140-340ml/hr) while the second one corresponds to regions with relatively low flow rates (*case 2*: 20-60ml/hr). In both cases the pulse frequency is 1Hz, a rate that corresponds to 60 heart strokes per minute. For these flow conditions the Reynolds (*Re*) number ranges from 9 to 21 (*case 1)* and from 1.1 to 3.5 (*case 2)*. An important quantity to be taken into account in pulsatile flow is Womersley number, which is the ratio of transient inertia over viscous forces and is defined as $\alpha = R\sqrt{\omega\rho / \mu}$, where ω is the angular frequency, ρ the density and μ the dynamic viscosity. For the present study Wormesley number retains a constant value (α=0.42), because both the diameter of the micro channel and the frequency of the pulse are constant.

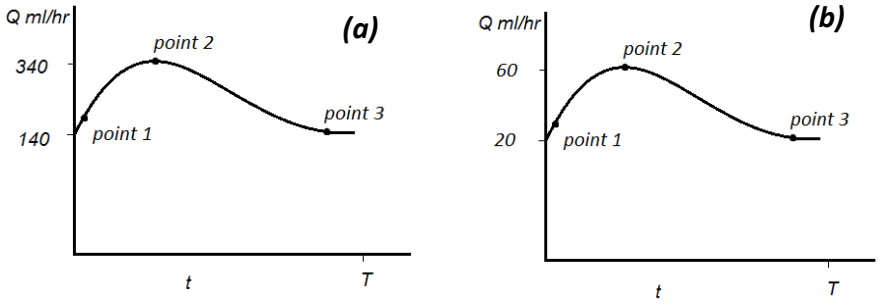

Figure 5: Schematic of pulse employed for: a) high flow rate and b) low flow rate.

All experiments were conducted in a 600μm hydraulic diameter micro channel (matching arteriole dimensions) manufactured by laser ablation in a polymeric chip and sealed with the same material. As the construction of a cylindrical micro channel is very difficult the cross section of the experimental module is trapezoidal and this is not far from the asymmetric geometry of human arteries. The geometric characteristics of the experimental region are presented in ***Figure 6***. The velocity data were acquired from the middle plane of the bifurcation, i.e. 300μm over the channel bottom.

A backlight *μ-PIV* system was used to study the flow. A micro strobe emitting at 532nm was used to illuminate the measurement section of the micro channel. The flow was recorded with a high sense *CCD* camera *(Hisense MkII, 1.4 Mpixel, 70% quantum efficiency),* coupled to a Nikon microscope *(Eclipse LV100)*. Polystyrene particles with mean diameter of 1μm were added to the fluids for tracing the flow. In order to obtain magnified images a 10X air immersion objective with NA=0.30, was used. Time delays between frames were ranging from 100 to 350μs while the sampling rate was 5Hz (i.e. five mea-

surements during each pulse were taken). The image processing and the velocity estimations were performed using *Flow Manager* Software (by *DantecDynamics*). A typical velocity measurement at the middle plane of the bifurcation is shown in ***Figure 7.***

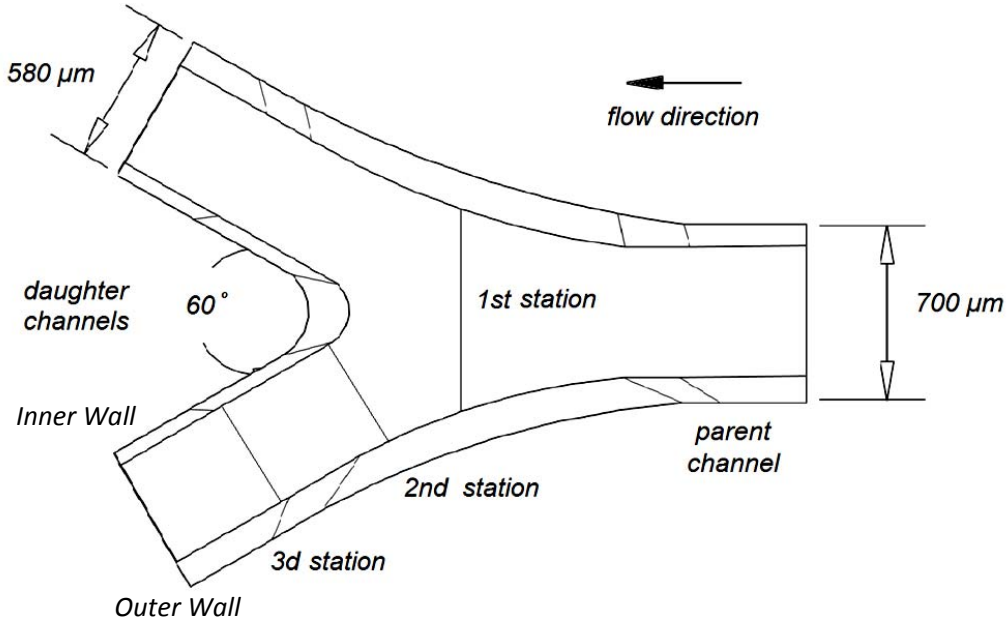

Figure 6: Bifurcation geometry and three positions of measurements.

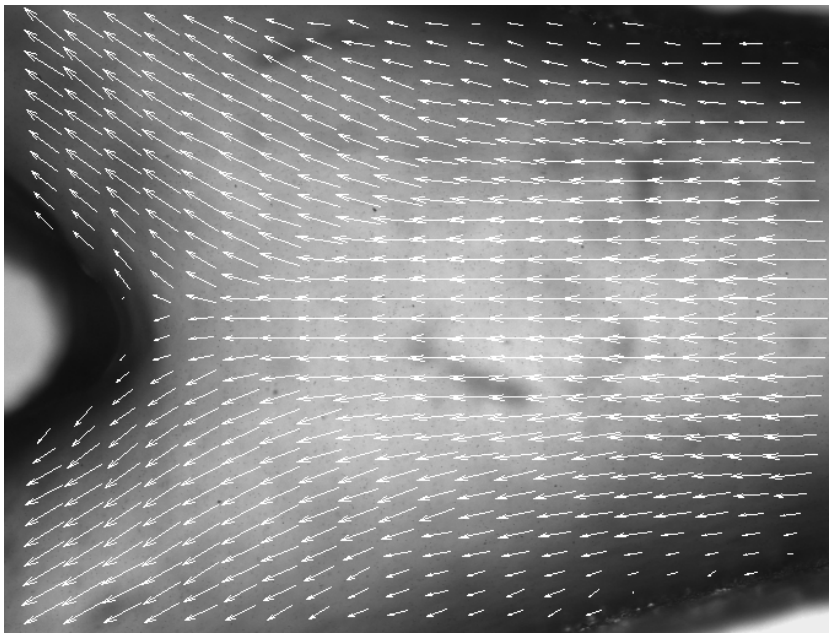

Figure 7: Typical velocity measurement at the middle plane of the bifurcation.

To recapitulate, the following experimental conditions have been employed in order to simulate *in vivo* flow conditions:

- An aqueous glycerin solution containing a small amount of xanthan gum simulates the viscoelastic properties of blood.
- The flow is pulsatile.
- The walls of the micro channel are rigid while the cross section of the micro channel is trapezoid.

Results

In ***Figure 8*** the velocity distribution at *station 1* (at the parent channel) is presented for both the Newtonian and the non-Newtonian blood analogue. The latter exhibits a flat velocity profile, as expected.

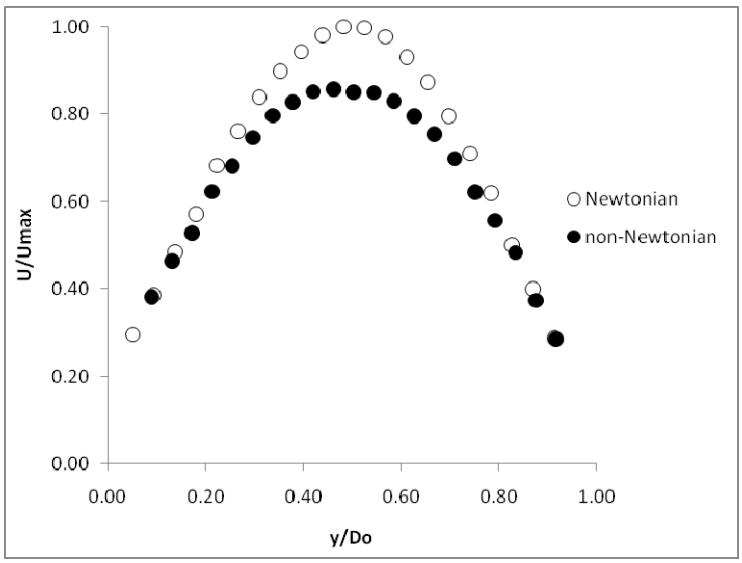

Figure 8: Normalized velocity profiles for the Newtonian and non-Newtonian fluids at *station 1*.

For the flow conditions of *case 1* it was observed that at *station 2* the position of the maximum axial velocity is shifted towards the inner channel wall. It is well known that in curved conduits, transverse secondary flows arise as a result of the interplay between centrifugal and viscous forces. Since the driving centrifugal force depends quadratically on the average velocity, while the viscous force depends linearly on average velocity, secondary flows are strongly suppressed for low velocities. According to Paras (1979), who conducted

experiments to simulate flow in human airways, for *Re<30* the secondary flow decays before the first diameter downstream the junction. This is verified by observing ***Figure 9***, where the velocity profile is parabolic at *station 1,* the maximum velocity is shifted after entering the bifurcation (*station 2*) and finally returns to the initial parabolic profile after a length of ***one*** diameter (*station 3*). Paras (1979) suggests that for such low *Re* the third velocity component (perpendicular to the main axis of the flow) is negligible and does not practically affect the velocity profiles measured in the two dimensional plane.

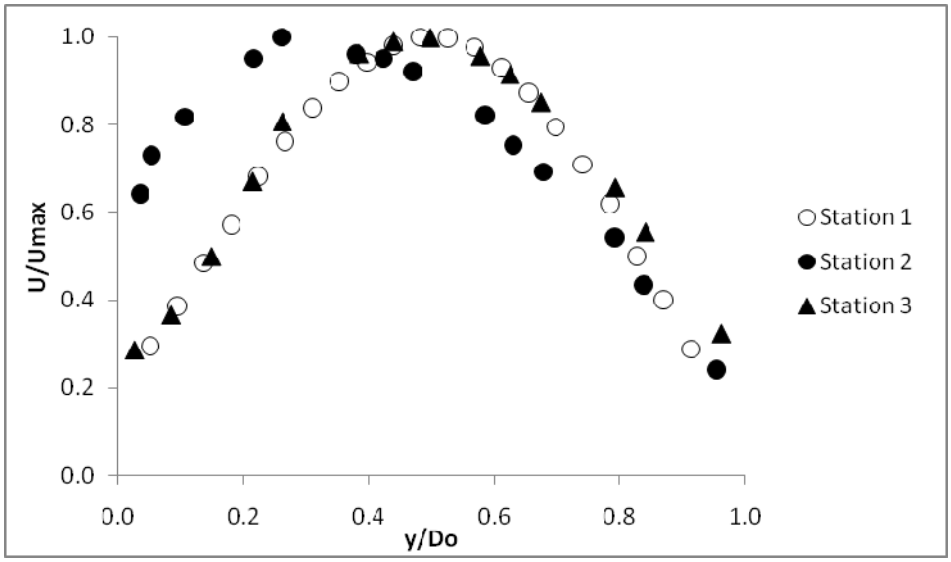

Figure 9: Normalized velocity profiles for the Newtonian fluid at *stations 1, 2* and *3*.

Figure 10 presents velocity measurements for *case 1* in the entrance of the bifurcation (*station 2*) for both fluids tested (i.e. Newtonian and non-Newtonian) at three instants of the pulse for *Re* from 9 to 21. The velocity data are normalized with respect to the maximum velocity of the corresponding fluid. It is obvious that the velocity distributions are different. For the Newtonian fluid the maximum velocity is shifted from the centerline towards the inner wall of the bifurcation, during the whole pulse cycle. This displacement is not that intense in the case of non-Newtonian fluid, which returns to a parabolic profile to the end of pulse.

In ***Figure 11*** the velocity profiles for *case 2* are presented. Measurements refer to the entrance of the bifurcation (*station 2*). The *Re* number for these flow conditions ranges between 1.4 and 3.5. As before, the velocity data are normalized with respect to the maximum

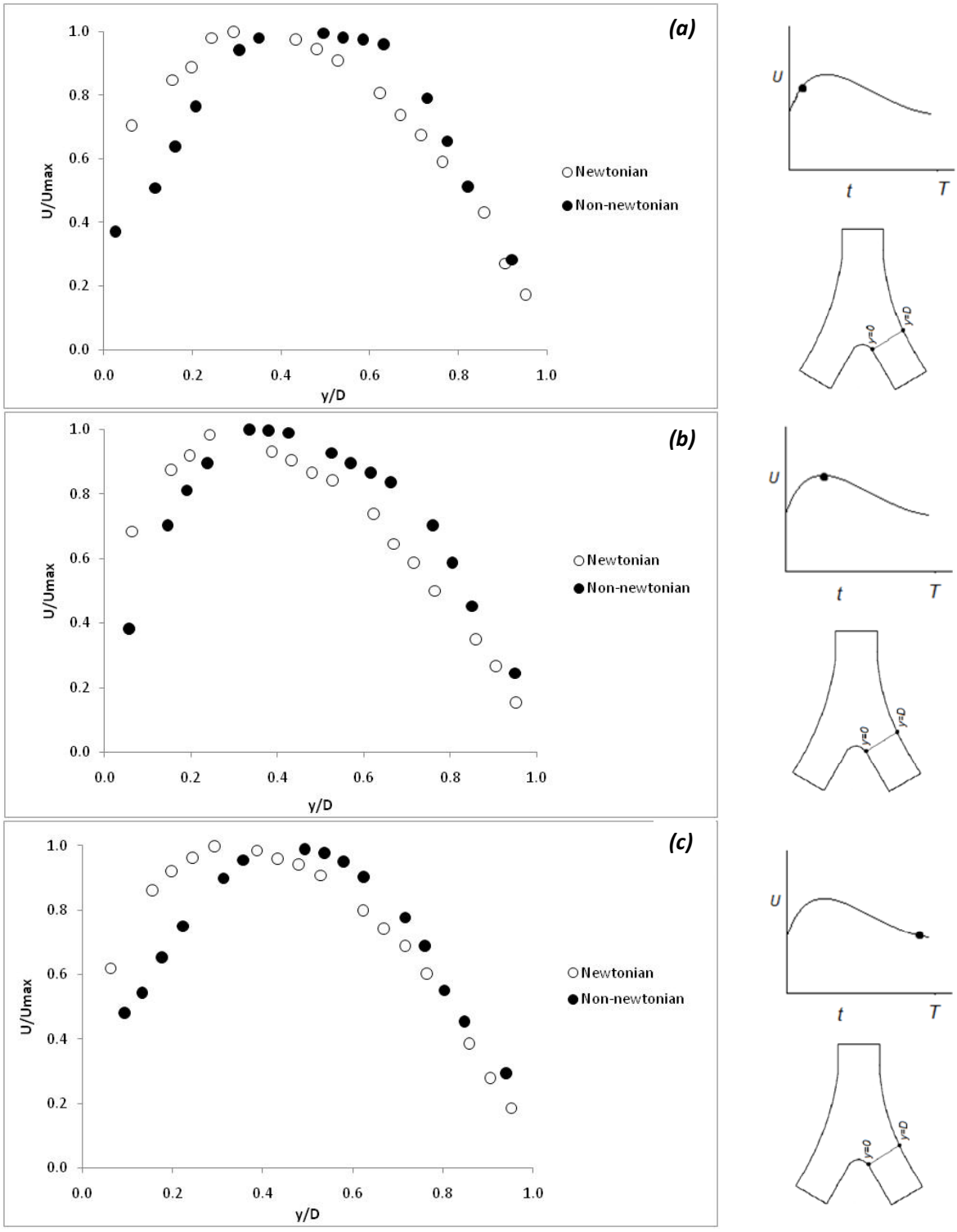

Figure 10: Normalized velocity profiles for Newtonian and non-Newtonian fluid (*case 1*).

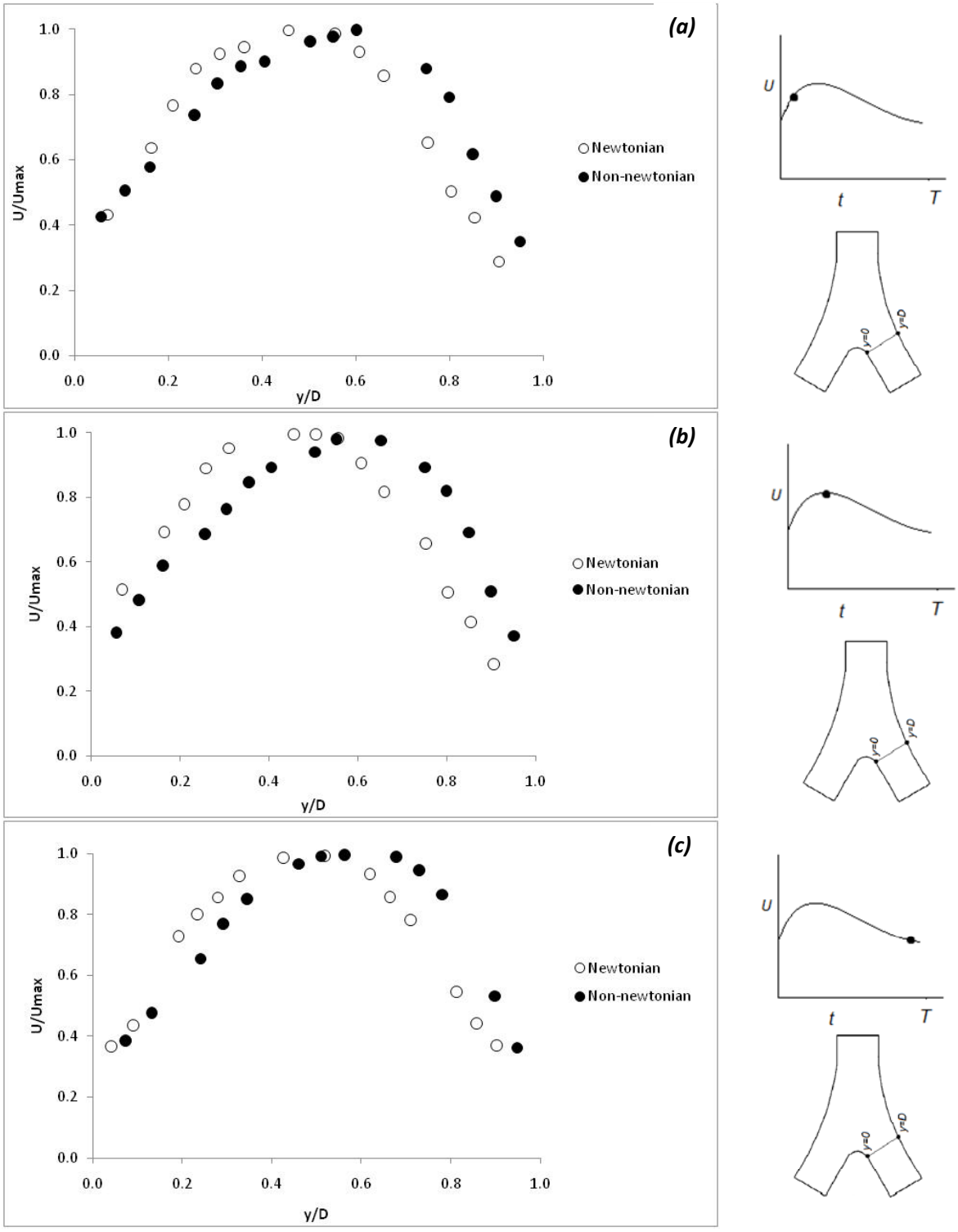

Figure 11: Normalized velocity profiles for Newtonian and non-Newtonian fluid (*case 2*).

velocity of the corresponding fluid. Comparing the distributions for the two fluids they are found to be quite different. Although both profiles are parabolic during a full pulse, the non-Newtonian fluid exhibits a flat velocity profile, as expected.

As mentioned above, the velocity data near the wall are utilized to provide estimation for the shear rate and consequently for the wall shear stress. In ***Figure 12*** the normalized shear stress, with respect to the maximum shear stress of the ***Newtonian analogue*** (τ_{wNmax}), on the inner wall of the bifurcation during a whole pulse for both fluids and for *case 1* is presented. As it can be seen the wall shear stress is ***lower*** for the non-Newtonian fluid and this difference ranges from 30 up to 40% depending on the instant of the pulse. This relies to the fact that, in *case 1* the Newtonian fluid shifts the maximum of the axial velocity towards the inner wall. Consequently, higher velocities and shears rates are occurred.

In ***Figure 13*** the difference between the estimated dimensionless wall shear stress for the inner and the outer wall for the two fluids is shown. In both cases the stresses on the outer wall are lower and that makes this area predisposed to plaque formation.

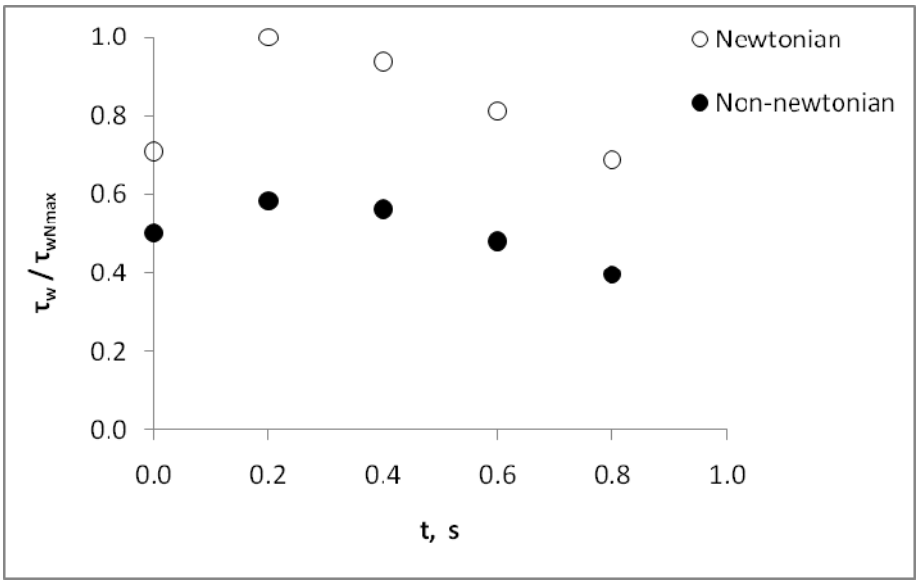

Figure 12: Normalized wall shear stresses at the inner wall of the bifurcation during a pulse (*case 1).*

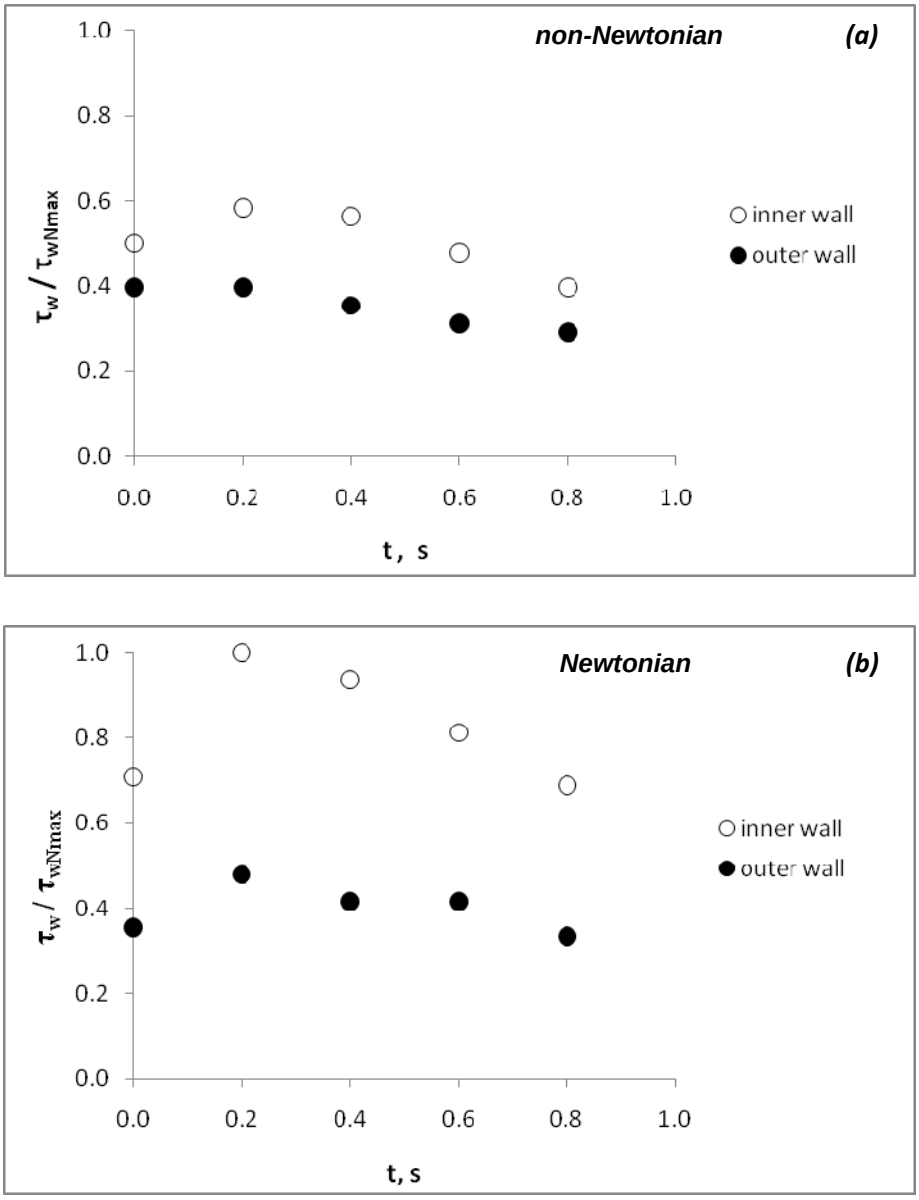

Figure 13: Normalized wall shear stresses at the inner and the outer wall for *case 1:* a) non-Newtonian fluid; b) Newtonian fluid.

Conclusions

The scope of this work is to study the pulsatile flow of blood at relatively low Reynolds numbers in a micro channel that simulates a bifurcated arteriole. The common assumption that blood behaves as Newtonian fluid was tested out. To summarize:

- The velocity profiles of the two fluids tested for both cases studied (high and low flow rate), as expected, are different. At the entrance of the bifurcation

the maximum of the profile is shifted towards the inner wall and thus the shear stresses there are higher than those on the outer wall. This is in agreement with relevant studies in larger human arteries and it means that the outer wall of the arteriole is more vulnerable to atherosclerosis than the inner one.

- It must be noted that the use of the Newtonian fluid gives raise to ***higher*** (30 to 40%) wall shear stress values in the bifurcation.

In conclusion, the assumption of the Newtonian behavior of blood does not hold true for low *Re* number flow in small blood vessels. It is also verified that the shear thinning behavior of blood significantly affects the velocity profiles and consequently the wall shear stress and the hemodynamic forces. The role of the latter is crucial in the understanding, diagnosis and treatment of cardiovascular diseases.

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