Diagnostic Uncertainties During Assessment of Serial Coronary Stenoses: An In Vitro Study

Currently, the diagnosis of coronary stenosis is primarily based on the well-established functional diagnostic parameter, fractional flow reserve (FFR: ratio of pressures distal and proximal to a stenosis). The threshold of FFR has a “gray” zone of 0.75–0.80, below which further clinical intervention is recommended. An alternate diagnostic parameter, pressure drop coefficient (CDP: ratio of trans-stenotic pressure drop to the proximal dynamic pressure), developed based on fundamental fluid dynamics principles, has been suggested by our group. Additional serial stenosis, present downstream in a single vessel, reduces the hyperemic flow, \( \dot{Q}_h \), and pressure drop, \( \Delta p \), across an upstream stenosis. Such hemodynamic variations may alter the values of FFR and CDP of the upstream stenosis. Thus, in the presence of serial stenoses, there is a need to evaluate the possibility of misinterpretation of FFR and test the efficacy of CDP of individual stenoses. In-vitro experiments simulating physiologic conditions, along with human data, were used to evaluate nine combinations of serial stenoses. Different cases of upstream stenosis (mild: 64% area stenosis (AS) or 40% diameter stenosis (DS); intermediate: 80% AS or 55% DS; and severe: 90% AS or 68% DS) were tested under varying degrees of downstream stenosis (mild, intermediate, and severe). The pressure drop-flow rate characteristics of the serial stenoses combinations were evaluated for determining the effect of the downstream stenosis on the upstream stenosis. In general, \( \dot{Q}_h \) and \( \Delta p \) across the upstream stenosis decreased when the downstream stenosis severity was increased. The FFR of the upstream mild, intermediate, and severe stenosis increased by a maximum of 3%, 13%, and 19%, respectively, when the downstream stenosis severity increased from mild to severe. The FFR of a stand-alone intermediate stenosis under a clinical setting is reported to be \( \sim 0.72 \). In the presence of a downstream stenosis, the FFR values of the upstream intermediate stenosis were either within (0.77 for 80%–64% AS and 0.79 for 80%–80% AS) or above (0.88 for 80%–90% AS) the “gray” zone (0.75–0.80). This artificial increase in the FFR value within or above the “gray” zone for an upstream intermediate stenosis when in series with a clinically relevant downstream stenosis could lead to misinterpretation of functional stenosis severity. In contrast, a distinct range of CDP values was observed for each case of upstream stenosis (mild: 8–10; intermediate: 47–54; and severe: 130–155). The nonoverlapping range of CDP could better delineate the effect of the downstream stenosis from the upstream stenosis and allow for the accurate diagnosis of the functional severity of the upstream stenosis. [DOI: 10.1115/1.4026317]

Keywords: serial stenoses, coronary diagnostic parameters, fractional flow reserve, pressure drop coefficient, in vitro experiments

1 Introduction

Coronary artery disease is known to be a common cause of death among the U.S. population. It may be initiated by a single focal stenosis or multiple stenoses in series or a diffuse atherosclerotic narrowing, either present in a single epicardial vessel or multiple vessels [1,2]. A disease in the downstream microvasculature could also be present in addition to an epicardial stenosis. Quantitative coronary angiography has been a common standard, extensively used to visually evaluate the severity of a stenosis. However, the method of visual interpretation from an angiogram fails to assess the functional (hemodynamic) severity of a stenosis [3]. Therefore, current decisions for the treatment of coronary stenosis are based on well-established functional diagnostic parameters: coronary flow reserve (CFR) and fractional flow reserve (FFR) [4–6].

FFR is defined as the ratio of the pressures distal and proximal to a stenotic region [4]. FFR is the most widely used functional parameter and is considered by many to be the gold standard for the evaluation of functional significance of coronary stenosis [7–9]. Under a clinical setting, FFR is invasively measured with a pressure wire at maximal arterial vasodilation (hyperemia) induced using a vasodilatory drug. Previous clinical studies have reported an FFR cut-off of 0.75 for a stenosis present in a single vessel [4,7,10] and 0.80 for stenoses present in multiple vessels [11–13]. Cases with an FFR below 0.75 are recommended for further clinical intervention while those with a value above 0.80 are deferred further intervention. The region between 0.75 and 0.80 is known to be the “gray” zone for which diagnosis remains uncertain. Thus, another diagnostic parameter, CFR, invasively measured with a pulsed Doppler catheter, is defined as the ratio of the flow at hyperemia to the flow at resting (basal) condition [14,15]. A CFR value of \(< 2.0\) indicates a condition of either epicardial dysfunction or microvascular impairment [5]. However, during in vitro testing, CFR and FFR can be evaluated using non-invasive measurements of flow and pressure,
obtained using a flow sensor and pressure scanner, respectively.

CFR, which is based on flow alone, fails to delineate between an epicardial and microvascular disease and is affected by fluctuations in hemodynamic factors such as heart rate, blood pressure, and myocardial contractility [16,17]. FFR, on the contrary, is known to remain unaffected by these hemodynamic factors [18]. However, it is known to be affected by changes in hemodynamic conditions brought about by the presence of either downstream serial stenoses or concomitant microvascular disease or downstream collaterals [19–21]. Therefore, we have proposed an alternate diagnostic parameter, pressure drop coefficient (CDP), derived from fundamental fluid dynamics principles. CDP is defined as the ratio of the trans-stenotic pressure drop to the dynamic pressure proximal to a stenosis [22]. Simultaneous measurements of functional parameters (pressure drop and proximal velocity), necessary for the evaluation of CDP, can be readily obtained during routine cardiac catheterization procedures using a dual-sensor tipped guidewire, also known as a combowire [23–27]. CDP has been validated in pre-clinical trials [21,23,24,26,27] and under a clinical setting [25], in order to delineate epicardial stenoses. Furthermore, it has been shown to be independent of heart rate [24,26] and contractility [27,28] in recently conducted in vivo animal studies. CDP is known to have a large range of values comprising a lower and upper limit of 0 and 1000. A specific diagnostic cut-off value is currently under clinical investigation.

Evidence of additional stenoses present in a single vessel is often observed in many clinical scenarios [29–34]. In the presence of serial stenoses, the additive nature of the resistances of the individual stenoses would lead to an increase in the pressure drop, Δp, coupled with a reduction in hyperemic flow, Qh, in the coronary vessel [35,36]. Conventional angiographic assessment of serial stenoses severity is based only on the degree of area reduction, ignoring the hemodynamic complexities and changes caused by multiple stenoses in a single vessel [1]. Conclusions from several in vitro and in vivo tests indicate that serial stenoses have a greater effect on the hyperemic flow response than a single stenosis of equivalent length and severity [35,37–41]. Such observations emphasize the need for better assessment of serial coronary stenoses.

The evidence of the complex fluid dynamic interaction between serial stenoses has been reported in literature [1,32,33,35,36,42]. Therefore, the need to evaluate these interactions while assessing the severity of the individual stenosis is of clinical importance to the cardiovascular intervention community. In the presence of serial stenoses, the clinical diagnosis and treatment of disease is focused on conducting angioplasty on the more significant stenosis, thus failing to evaluate the individual stenosis since one stenosis may affect the hemodynamic significance of the other. A second stenosis, present downstream, increases the resistance of the coronary vessel, thereby reducing Qh and, consequently, Δp across an upstream stenosis and vice versa. More importantly, a stenosis located downstream is shown to have a greater hemodynamic effect on the one located upstream [32,33]. The true severity of an upstream stenosis may thus be masked by the presence of a downstream stenosis, particularly if the severity of the downstream stenosis is comparable to or greater than that of the upstream stenosis.

CFR is restricted to assessing the combined functional severity of serial stenoses, thus failing to evaluate the individual stenosis severity. On the contrary, pressure-based FFR could be used to assess the functional severity of the individual stenosis, but it may be overestimated in the presence of serial stenoses because of the decrease in Qh and Δp. Thus, FFR may fail to accurately interpret the true functional severity of individual stenoses in series, leading to the misinterpretation and misdiagnosis of a clinically relevant stenosis.

### Table 1 Dimensions of stenotic geometries used

<table>
<thead>
<tr>
<th>Case</th>
<th>l(r) (mm)</th>
<th>l(m) (mm)</th>
<th>d(m) (mm)</th>
<th>Δ Dp (mm)</th>
<th>% AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>6.28</td>
<td>0.39</td>
<td>1.59</td>
<td>0.98</td>
<td>3.0</td>
</tr>
<tr>
<td>Intermediate</td>
<td>6.35</td>
<td>0.95</td>
<td>1.62</td>
<td>1.32</td>
<td>3.0</td>
</tr>
<tr>
<td>Mild</td>
<td>6.96</td>
<td>3.15</td>
<td>1.79</td>
<td>1.75</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Here, AS denotes area stenosis.*

In order to address the effect of serial stenoses, De Bruyne et al. [29] revised the formulation of FFR to include the coronary wedge pressure in addition to the proximal and distal pressures. The revised FFR provided a better estimate of the true functional severity of individual stenoses located in series [33]. However, since its calculation involves the use of coronary wedge pressure that could be recorded only during coronary balloon angioplasty, the use of the revised FFR is merely limited to theory.

Furthermore, previous studies have failed to account for the reduction in Qh, an effect of serial stenoses on coronary autoregulation. Here, in order to delineate the effect of the downstream stenosis from the upstream stenosis, we assessed the Δp − Qh characteristics of serial stenoses using in vitro experiments while applying physiologic hemodynamic conditions. The extent of misinterpretation of the FFR values for the upstream stenosis was evaluated in the presence of a varying degree of downstream stenoses. In addition, the alternate diagnostic parameter, CDP, was evaluated to determine the functional severity of the individual stenosis in the presence of serial stenoses.

### 2 Methodology

The following section describes the in vitro experimental setup along with the detailed protocol used for assessing the effect of serial stenoses under physiologic conditions of flow and pressure.

#### 2.1 Stenosis Geometry and Test Section

Angiographic evidence from an in vivo data set of 32 patients [43] was used to construct rigid models of arterial stenosis representing a mild (~64% area stenosis (AS) or a 40% diameter stenosis (DS)) and severe (~90% AS or 68% DS) condition [44–46]. A third stenosis of intermediate severity (~80% AS or 55% DS) was constructed using clinical data reported by Roy et al. [46] and Back and Denton [47]. The stenosis models, fabricated from Lexan material, were assumed to have a concentric shape with smooth walls. The geometric dimensions of the three stenoses are tabulated in Table 1. Each stenosis was composed of a converging and diverging section separated by a throat region. The three individual stenoses were paired in series in various combinations to form serial stenoses. A typical test section with the two serial stenoses (see Fig. 1(a)) consisted of an upstream and downstream stenosis connected with a Plexiglas connector. Nine combinations of serial stenoses were obtained by varying the severity (mild, intermediate, and severe) of both the upstream and downstream stenoses. The proximal and distal regions represented the inlet and outlet of each stenosis (see Fig. 1(a)). A constant distance was maintained between the two stenoses for all of the combinations in order to assess only the primary effect of area reduction on the diagnostic parameters, thus neglecting the secondary effect of length.

#### 2.2 Experimental Setup

The flow loop, shown in Fig. 1(b), was used to perform benchtop tests on serial stenoses under physiologic conditions of flow and pressure. The blood analog fluid (BAF), circulated through the loop, was composed of 80% water, 20% glycerin, and 0.02% xanthum gum by weight, in order to closely mimic the non-Newtonian behavior of blood. The viscosity of the BAF fluid was measured using a concentric cylinder viscometer (Brookfield Engineering Laboratories Inc.), and then matched with the standard Carreau model, similar to our previous studies [48,49]. A pulsatile blood pump (Harvard Apparatus) was...
used to generate ventricular flow. The flow at the outlet of the pump was split into two branches, one representing aortic flow and the other representing flow through the coronary circulation, which contained the serial stenoses test-sections. A physiologic diastolic-dominant coronary flow pulse was then obtained by adjusting the flow-level in the compliance chambers, Comp1 and Comp2, and regulating the flow rate using the flow valves, R1 and R2 (see Fig. 1(b)). An ultrasound Doppler flow sensor connected to a digital flow meter console (Transonic Systems Inc.) measured the flow rate at the inlet of the test section. The variation in pressure across the test sections was recorded using eight pressure ports spread over the length of each of the individual stenoses placed in series. A total of 16 ports were connected to a pressure sensor array module (Scanivalve Corp.), calibrated up to an accuracy of ±0.20%. The simultaneous recording of instantaneous flow and pressure was achieved through a data acquisition system fitted with an analog input module (National Instruments). Post-processing of the pressure and flow data was done using user-defined macros in Microsoft Excel.

### 2.3 Pressure and Flow Measurements

An example of the flow pulse and pressure pulses proximal to the upstream and downstream stenosis obtained during the experiment is shown in Fig. 2. These pulses are consistent with previous studies [45,49–51]. Apart from the mean pressure values, no significant difference was observed between the shapes of the two pressure pulses. The time period of the flow pulse in all cases was approximately 0.8 s; thus representing a typical cardiac cycle. The ratio of the average to peak flow rate varied in the range of 0.5–0.7 [45,51]. Based on clinical evidence, mean aortic pressures ($\overline{p_a}$) were maintained at 89 mm Hg, 86 mmHg, and 84 mm Hg for an upstream stenosis with severe, intermediate, and mild area blockage, respectively [43,47].

A 0.014 in. (0.35 mm) guidewire was inserted across the serial stenoses with its tip located in the distal region of the downstream stenosis in order to replicate a clinical setting. This configuration is referred to as condition C1. Pressure recordings were made at four different flow rates for each serial stenoses combination. The guidewire was then pulled back across the downstream stenosis such that the tip was then located in the distal region of the upstream stenosis. This configuration is referred to as condition C2. Similar pressure-flow recordings were repeated under this configuration for all of the nine combinations of serial stenoses.
Three sets ($n = 3$) of experiments were carried out and the three pressure-flow data sets were averaged to obtain the pressure drop-flow rate ($\Delta p - \dot{Q}$) curves for each of the nine combinations of serial stenoses.

### 2.4 Diagnostic Parameters

The diagnostic parameters, fractional flow reserve (FFR) and pressure drop coefficient (CDP), were evaluated at hyperemia for assessing the severity of the individual stenosis. In the following sections, the functional stenosis severity assessed using FFR and CDP is referred to as stenosis severity. A brief overview of these parameters is given in Secs. 2.4.1 and 2.4.2.

#### 2.4.1 Fractional Flow Reserve

FFR is defined as the ratio of maximum myocardial blood flow in the presence of a stenosis to the theoretical maximum flow in a normal artery. Under physiologic condition, at hyperemia, the resistance offered by the distal microvasculature is considered minimal and the blood flow is proportional to the driving pressure. Therefore, the FFR can be calculated as the ratio of the pressures distal and proximal to a stenosis [4]

$$FPR = \frac{\tilde{p}_D - \tilde{p}_C}{\tilde{p}_D - \tilde{p}_C}$$

where $\tilde{p}_D$ (mm Hg) and $\tilde{p}_C$ (mm Hg) are the mean hyperemic pressures recorded in the proximal and distal regions of a stenosis, respectively. Here, $\tilde{p}_C$ (mm Hg) is the mean venous pressure which has a value of $\sim 0$ mm Hg, signifying negligible microvascular impairment.

#### 2.4.2 Pressure Drop Coefficient

CDP is defined as the ratio of the trans-stenotic pressure drop to the dynamic pressure proximal to a stenosis [22]. CDP has been derived based on the fundamental principles of fluid dynamics and signifies the dimensionless pressure drop across a stenosis [52,53]

$$CDP = \frac{\Delta \tilde{p}}{0.5 \rho U_e^2}$$

where $\Delta \tilde{p}$ (dyne/cm$^2$) represents the mean hyperemic pressure drop across a stenosis. Here, $U_e$ (cm/s) is the mean hyperemic velocity recorded in the proximal region of a stenosis and $\rho$ (g/cm$^3$) is the fluid density.

### 3 Results

The pressure drop-flow rate ($\Delta \tilde{p} - \dot{Q}$) characteristics, derived from the in vitro experiments, were used to construct the coronary flow reserve-distal perfusion pressure (CFR-DFP) plot in order to obtain physiologic hyperemic flow rates for different combinations of serial stenoses. Subsequently, the effect of a varying degree of downstream stenosis on the hyperemic flow, $\dot{Q}_h$, and $\Delta \tilde{p}$ across an upstream stenosis was evaluated. In addition, the severity of the upstream stenosis was assessed using diagnostic parameters; FFR and CDP. Data sets for all of the serial stenoses’ combinations obtained for condition C1, where the guidewire is placed across both stenoses (i.e., the sensor tip is located in the distal region of the upstream stenosis) showed similar trends and produced similar outcomes when compared to those obtained under condition C1. Hence, a brief analysis of the results obtained under condition C2 has been included in the Appendix section.

#### 3.1 Pressure Drop-Flow Rate ($\Delta \tilde{p} - \dot{Q}$) Characteristic Curves

The $\Delta \tilde{p} - \dot{Q}$ characteristics of serial stenoses can be expressed by a quadratic relation $\Delta \tilde{p} = aQ + b\dot{Q}^2$ obtained after a polynomial curve-fit to the $\Delta \tilde{p} - \dot{Q}$ data points [37,42,51–54]. The effects of viscous loss and loss due to the change in momentum, contributing to the pressure drop across serial stenoses, are represented by the terms $a\dot{Q}$ and $b\dot{Q}^2$, respectively. The corresponding contribution of each effect can be weighed through the viscous loss coefficient ‘$a$’ and the loss coefficient ‘$b$’ due to the change in momentum.

Figure 3 shows the $\Delta \tilde{p} - \dot{Q}$ curves of the nine combinations of serial stenoses obtained from in vitro experiments under conditions C1 and C2. For both conditions, each $\Delta \tilde{p} - \dot{Q}$ curve was plotted using the total $\Delta \tilde{p}$, which was obtained using the pressure ports and by summing the pressure drops across the individual stenoses placed in series [1,5,35,42,55]. A physiologic limiting $\Delta \tilde{p}$ of 35 mm Hg was imposed in order to account for the Brown–Bolton–Dodge criteria for subendocardial ischemia [56]. Figure 3(a) represents the $\Delta \tilde{p} - \dot{Q}$ curves obtained when the guidewire was placed across both stenoses (condition C1). The $\Delta \tilde{p} - \dot{Q}$ curves obtained when the guidewire was placed across the upstream stenosis only (condition C2) are shown in Fig. 3(b). In general, with the increase in severity of serial stenoses (upstream or downstream or both) from mild to severe, the nonlinearity of the $\Delta \tilde{p} - \dot{Q}$ curve increased, as indicated by higher values of the ‘$b$’ coefficient (see Fig. 3). The method to obtain physiologically relevant $\Delta \tilde{p}$ and $Q$ values at hyperemia using the experimental $\Delta \tilde{p} - \dot{Q}$ curves is explained in Sec. 3.2.
3.2 Determination of Hyperemia Using Coronary Flow Reserve-Distal Perfusion Pressure and $D_p/C_0$ Plots. The hyperemic flow rates, $Q_h$, for all of the serial stenoses combinations were obtained using the intersection of the coronary flow reserve-distal perfusion pressure (CFR-$D_p$) line and the experimental $D_p/C_0$ curve (see Fig. 4) [46,48,51]. The linear curve in Fig. 4 represents the relationship between the CFR and the corresponding $D_p$ of 32 patients [43] having a focal lesion and undergoing percutaneous transluminal coronary angioplasty (PTCA). Evidence of any microvascular dysfunction was not reported in these patients. The left ventricular ejection fraction remained normal with no left ventricular hypertrophy or valvular heart disease. In addition, no signs of myocardial infarction or stenosis in the parent branch or angiographically evident collateral flow were observed. As seen in Fig. 4, the CFR-$D_p$ line has an $x$-intercept of 20 mm Hg. At this zero-flow pressure $p_d$ flow to the subendocardium ceases. Van Herck et al. [57] observed a $p_d$ of 14.6 ± 8.0 mm Hg in patients with stable angina pectoris whereas Bache and Schwartz [58] reported a $p_d$ of 18 mm Hg for a dog. The distal perfusion pressure at hyperemia $p_d$ (the $x$-axis of Fig. 4) of serial stenoses was obtained using the experimental obtained $\Delta p - Q$ characteristic curve of the serial stenoses in combination with the physiologically reported linear CFR-$p_d$ plot provides the $p_d$ at the downstream stenosis and the CFR ($Q_h/Q_b$ (the $y$-axis in Fig. 4)); where $Q_h$, is the hyperemic flow and $Q_b$ is the known value of basal flow) [46,54,59]. In this study, a $Q_b$ value of 50 ml/min was considered for the calculation of the CFR [43].

Figure 4(a) indicates the estimation of $Q_h$ under condition C1. For example, in case of the 90%–64% AS combination, the intersection of the $\Delta p - Q$ characteristic curve and the CFR-$p_d$ line provides a CFR value of 2.1 and a $p_d$ of 52.5 mm Hg (the dotted horizontal and vertical lines in Fig. 4(a)). Keeping the upstream % AS to be severe (90% AS or 68% DS), the CFR decreased from 1.9 to 1.5 when the downstream stenosis severity was increased from intermediate (80% AS or 55% DS) to severe (90% AS) (see arrow number 1 in Fig. 4(a)). Consequently, the corresponding $p_d$ decreased from 50.1 mm Hg to 43.6 mm Hg. For the case with an upstream stenosis of intermediate severity (80% AS), the CFR decreased from 2.7 to 2.5 and, subsequently, to 1.8 when the severity of the downstream stenosis was increased from mild to intermediate and, subsequently, to a severe % AS, respectively. Consequently, the $p_d$ for the corresponding cases decreased from 62.3 mm Hg to 59.1 mm Hg and, subsequently, to 48.1 mm Hg. Similarly, when the upstream stenosis severity was mild (64% AS or 40% DS), the CFR decreased from 3.3 to 2.6 and further to 1.9 when the severity of the downstream stenosis was increased from mild to intermediate and, further, to a severe % AS, respectively. A subsequent decrease in $p_d$ from 71.5 mm Hg to 60.0 mm
Table 2  Effect of serial stenoses on flow and pressure

<table>
<thead>
<tr>
<th>Downstream stenosis</th>
<th>Hyperemic flow, (Q_h) (ml/min)</th>
<th>Upstream stenosis pressure drop, (\Delta p_u) (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>64%</td>
<td>80%</td>
</tr>
<tr>
<td>Upstream stenosis</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>90%</td>
<td>104.8</td>
<td>106.1</td>
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<tr>
<td>80%</td>
<td>136.4</td>
<td>134.6</td>
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<tr>
<td>64%</td>
<td>166.1</td>
<td>170.7</td>
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Table 3  Effect of serial stenoses on diagnostic parameters

<table>
<thead>
<tr>
<th>Downstream stenosis</th>
<th>FFR</th>
<th>CDP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>64%</td>
<td>80%</td>
</tr>
<tr>
<td>Upstream stenosis</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>90%</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>80%</td>
<td>0.77</td>
<td>0.76</td>
</tr>
<tr>
<td>64%</td>
<td>0.94</td>
<td>0.94</td>
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</tbody>
</table>

Hg and further to 49.7 mm Hg was observed for the respective cases.

In general, for each case of upstream stenosis, the CFR and \(p_{rh}\) values of the combination decreased with an increase in downstream severity. An overlap of CFR and \(p_{rh}\) values was observed between each case of upstream stenosis as the downstream severity was varied. Similar trends were observed in the CFR-\(p_{rh}\) values evaluated under condition C2 (see Fig. 4(b)).

3.3  Guidewire Across Both the Stenoses (Condition C1)

3.3.1  Effect of Serial Stenoses on Flow and Pressure. The bar graphs plotted in Figs. 5(a) and 5(b) indicate the effect of the presence of a downstream stenosis on the hyperemic coronary flow, \(Q_h\), and pressure drop across the upstream stenosis, \(\Delta p_u\), (the subscript ‘u’ denotes an upstream stenosis), respectively, when evaluated under condition C1 (see Table 2).

**Upstream severe stenosis.** The \(Q_h\) decreased from 104.8 ml/min to 97.1 ml/min when the downstream severity increased from mild to intermediate. As the downstream stenosis increased further to a severe condition, \(Q_h\) decreased to 76.1 ml/min; thus indicating an overall reduction of nearly 27% (percentage variation = (maximum value – minimum value) × 100/maximum value) in maximum flow across the upstream severe stenosis (see Table 2). A \(\Delta p_u\) of 33.5 mm Hg was observed across the upstream severe stenosis in the presence of a downstream mild stenosis. The \(\Delta p_u\) decreased to 27.7 mm Hg and further to 20.4 mm Hg in the presence of a downstream intermediate and severe stenosis, respectively. Due to the reduction in \(Q_h\), a significant overall reduction (~39%) in \(\Delta p_u\) was observed when the downstream stenosis severity increased from mild to severe.

**Upstream intermediate stenosis.** The \(Q_h\) decreased from 136.4 ml/min to 126.4 ml/min for cases with a downstream mild and intermediate stenosis, respectively (see Table 2). The value of \(Q_h\) corresponding to the case with a downstream severe stenosis was 90.7 ml/min. Thus, a greater overall reduction (~34%) in \(Q_h\) was observed for this case in comparison to the case with an upstream severe stenosis. Subsequently, \(\Delta p_u\) across the intermediate stenosis decreased from 19.7 mm Hg to 17.8 mm Hg and, further, to 10.1 mm Hg for the cases with a downstream mild, intermediate, and severe stenosis, respectively, resulting in a reduction of \(\Delta p_u\) of nearly 49%, considering the entire range.

**Upstream mild stenosis.** By varying the severity of the downstream stenosis, a significant effect was observed on \(Q_h\) and \(\Delta p_u\) for an upstream mild stenosis (see Table 2). The \(Q_h\) decreased by nearly 42% (166.1 ml/min for 64%–64% AS, 130.8 ml/min for 64%–80% AS, and 95.9 ml/min for 64%–90% AS) while the \(\Delta p_u\) decreased by nearly 57% (4.8 mm Hg for 64%–64% AS, 3.7 mm Hg for 64%–80% AS, and 2.1 mm Hg for 64%–90% AS).

In general, \(Q_h\) through a coronary vessel decreased when the severity of the downstream stenosis was increased. Consequently, \(\Delta p_u\) also reduced with an increase in the downstream % AS.

3.3.2  Effect of Serial Stenoses on Diagnostic Parameters. The diagnostic parameters, FFR and CDP (see Table 3), were calculated for the upstream stenosis using the hyperemic values of the previously discussed pressure and flow. The effect of serial stenoses, evaluated under condition C1 on the FFR and CDP is shown in Figs. 6(a) and 6(b), respectively. The shaded region of FFR from 0.75 to 0.80 in Fig. 6(a) corresponds to the “gray” zone of FFR for which diagnostic uncertainties are well documented. Clinical intervention is generally recommended for FFR values below 0.75.

**Fractional Flow Reserve (FFR)**

**Upstream severe stenosis.** FFR of the upstream severe stenosis was 0.62 when a mild stenosis was placed downstream (see Table 3). Similarly, an FFR value of 0.69 was recorded for the case with a downstream intermediate stenosis. This value increased to 0.77, which was within the “gray” zone, when a severe stenosis was placed downstream.

**Upstream intermediate stenosis.** The FFR values for a single intermediate stenosis were previously reported to be 0.72 [60] and 0.73 [51], based on clinical and in vitro studies, respectively. In the presence of a downstream mild and intermediate stenosis, the FFR values of the upstream intermediate stenosis increased to 0.77 and 0.79, respectively, and were within the “gray” zone (see Fig. 6(a)). Hence, the severity of the upstream intermediate stenosis may be misdiagnosed when placed in series with a downstream mild or intermediate stenosis. When the downstream severity increased further to a severe stenosis, the FFR value increased to 0.88. This value is well above the upper threshold limit for clinical intervention and could lead to the incorrect diagnosis, leading to...
misinterpretation and misdiagnosis of stenosis severity. While in the presence of a downstream severe stenosis, the severity increased by a maximum of 3%, 13%, and 19% for mild to severe stenosis, causing reduced hyperemic flow and irrespective of the FFR value. Under such a scenario, for the case with an upstream intermediate stenosis present in series with a downstream severe stenosis, the lesion will be re-assessed using the FFR under increased hyperemic flow conditions to determine the true severity of the upstream intermediate stenosis. Furthermore, the overestimation in the FFR of the upstream severe stenosis would not affect clinical decision making when present in series with another downstream severe stenosis.

Pressure Drop Coefficient (CDP)

Upstream severe stenosis. The CDP values of the severe stenosis corresponding to the cases with a downstream mild and intermediate stenosis were 134 and 129. However, the CDP value somewhat increased to 155 when a severe stenosis was placed downstream. Thus, the CDP showed no specific trend with the increase in the downstream stenosis severity (see Fig. 6(b)).

Upstream intermediate stenosis. The CDP of the intermediate stenosis was 47 when combined with a downstream mild stenosis, which then increased to a value of 49 when placed in series with a downstream intermediate stenosis. When the % AS of the downstream stenosis increased to severe, the CDP of the upstream stenosis further increased to 54 (see Table 3).

Upstream mild stenosis. An insignificant increase in the CDP values of the upstream mild stenosis was observed when the downstream severity was increased (8 for 64%–64% AS, 9 for 64%–80% AS, and 10 for 64%–90% AS).

Hence, minimal changes were observed in the CDP values of the upstream stenosis when the downstream severity was increased. More importantly, a distinct range of CDP values was observed for each case of upstream stenosis (mild: 47-54; intermediate: 47-54; and severe: 130-155).

4 Discussion

Variations in flow and pressure, caused by the presence of serial stenoses in a coronary artery, are observed to influence the diagnostic parameters: FFR and CDP. For serial stenoses, the increased values of the FFR for an upstream intermediate stenosis could be within the cut-off range of 0.75–0.80. Such an increase in the FFR near the “gray” zone may lead to misinterpretation of stenosis severity. Hence, in the present in vitro experiment, we assessed the variability of the FFR and CDP of upstream mild, intermediate, and severe stenosis, when in series with a downstream stenosis of different area obstruction. In the presence of a downstream stenosis, the FFR of a clinically relevant upstream intermediate and severe stenosis was overestimated above or near the clinical cut-off value, which may lead to misdiagnosis of stenosis severity. The CDP, on the contrary, showed a distinct range of values for each case of the upstream stenosis. This could allow for an accurate diagnosis of the upstream stenosis severity, irrespective of the presence of downstream stenoses.

Additional resistance offered by a downstream stenosis reduces hyperemic flow, \( \dot{Q}_h \) through the coronary vessel. As a consequence, the pressure drop across an upstream stenosis, \( \Delta p_u \), decreases. Such flow-related changes in \( \Delta p_u \), observed in this study, are comparable to previous studies on serial stenoses [32,33,35,36]. Since the FFR is based on the pressure ratio alone, the decrease in \( \Delta p_u \) would lead to a subsequent increase in the FFR values of the upstream stenosis. In order to analyze this increase in the FFR, in the presence of an additional downstream stenosis, a comparison of our results is conducted with data from a patient group having a single coronary stenosis [60]. The FFR of the single stenosis before intervention (78±10% AS), measured using a 0.014 in. guidewire, was 0.72±0.10, which then increased to 0.84±0.08 post-intervention (64±11% AS). An in vitro study previously conducted in our lab has reported an FFR value of 0.73 for a single intermediate stenosis, which is similar to the clinically observed value [51]. In the presence of an additional downstream stenosis, the FFR values of the upstream stenosis of intermediate

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**Fig. 6** Bar graphs indicating the effect of serial coronary stenoses on diagnostic parameters under condition C1: (a) FFR, and (b) CDP

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deferral of intervention for the clinically relevant intermediate stenosis.

**Upstream mild stenosis.** An overestimation in the FFR of the upstream mild stenosis was observed when the downstream stenosis severity increased from mild to intermediate and, further, to a severe area blockage (see Table 3). However, as observed in Fig. 6(a), all FFR values of the mild stenosis were above the “gray” zone [0.94 for 64%–64% AS, 0.96 for 64%–80% AS, and 0.98 for 64%–90% AS]. Under such a scenario, the functional diagnosis of serial stenoses remains unaffected.

Overall, with an increase in the severity of the downstream stenosis from mild to severe, causing reduced hyperemic flow and pressure drop, the FFR of the upstream mild, intermediate, and severe stenosis increased by a maximum of 3%, 13%, and 19%, respectively. For the clinically relevant upstream intermediate stenosis, while in the presence of a downstream severe stenosis, the FFR value increased above the threshold range. This may lead to misinterpretation and misdiagnosis of stenosis severity. Additionally, as previously discussed, for upstream severe and intermediate stenoses, uncertainties in the FFR values (the “gray” zone) could be observed when the severity of the downstream stenoses increased. However, in standard clinical practice, a visually severe stenosis would be treated based on angiographic evidence alone and irrespective of the FFR value. Under such a scenario, for the
(80% AS) and mild (64% AS) severities, evaluated under condition C1, were overestimated by a maximum of 18% of the pre-intervention and 14% of the post-intervention clinical values. Considering the reduction in $Qh$, such a moderate increase in the FFR for the upstream intermediate and mild stenoses, recorded in the presence of an additional downstream stenosis, is expected in a clinical setting.

Furthermore, in order to validate the results from our in vitro experiments, a comparison of our data is conducted with a clinical study that had patients having serial coronary stenoses within a single vessel [33]. Among all of the serial stenoses of the patient group, percutaneous transluminal coronary angioplasty (PTCA) was recommended for individual stenoses having ≥75% AS. The FFR of the upstream stenosis, measured with a 0.014 in. pressure guidewire, was 0.87 ± 0.08 in the presence of a downstream stenosis before the PTCA of the downstream stenosis. After the PTCA of the downstream stenosis, the FFR of the upstream stenosis decreased to 0.76 ± 0.02. This amounts to an overestimation of 14% in the FFR value of the upstream stenosis when its value is compared for pre- and post-PTCA of a downstream stenosis. This clinical data closely matches with: (a) the FFR $\sim 0.88$ calculated in the present study under condition C1, for the case with 80%–90% AS, which represents a pre-PTCA condition of the downstream stenosis, and (b) the FFR $\sim 0.76$ for 80%–64% AS representing a condition of post-PTCA of the downstream stenosis. Such a close comparison with clinical data provides confidence to the results of this study. More importantly, the elevated FFR observed for the upstream stenosis, before the PTCA of the downstream stenosis, indicates the possibility of misinterpretation and misdiagnosis of stenosis severity in the presence of serial stenoses.

In order to overcome the limitations of FFR for accurately diagnosing the severity of serial coronary stenoses, an alternate diagnostic parameter, CDP, is proposed [22]. CDP is unique since it includes the nonlinear $\Delta p - Q$ relationship developed from fundamental fluid dynamics principles. In addition, under hyperemic flow, the higher significance paid to the mean proximal velocity (square of $U_s$), can provide an increased ability to accurately resolve the delineation of an individual epicardial stenosis when placed in series. In general, as the severity of a single stenosis was varied, CDP was shown to change by a few orders (range: 0–1000) of magnitude [21,49,51,52]. Furthermore, CDP was shown to delineate the severity of epicardial stenosis under a clinical setting [25]. In the presence of a serial downstream stenosis, CDP values showed no specific trend or relative variation for the upstream severe stenosis, while the values for the upstream mild and intermediate stenosis showed an insignificant increasing trend with the increase in downstream severity, when evaluated under C1 condition. In contrast, considering a larger range of CDP, we observed distinct ranges of CDP values calculated for each upstream stenosis, irrespective of the variation in severity of the downstream stenosis. Such trends and the distinct ranges of CDP values for each case of upstream stenosis may indicate that the CDP is a stenosis-specific parameter which will delineate the effect of serial stenoses and better diagnose the severity of the individual stenosis.

In the present study, in vitro measurements of pressure and flow for the serial stenoses combinations were made under two conditions: one with the guidewire across both stenoses (condition C1) and the other with the guidewire across the upstream stenosis only (condition C2). The measurements made under condition C1 (in comparison to C2) provide a conservative estimate of $\Delta p$ since the presence of the guidewire across a stenosis causes a relatively higher reduction in flow, thus producing a somewhat decreased $\Delta p$ [46,61–63]. This limiting scenario would lead to the relatively higher overestimation in the FFR for serial stenoses. Furthermore, the results for the two conditions (C1 and C2 (see the Appendix)) did not alter the outcome.

4.1 Assumptions. The stenoses models were assumed to have a rigid wall based on clinical observations suggesting the loss of compliance in a stenosed artery [64,65]. In addition, the rigid wall assumption provides a conservative estimate of $\Delta p$ since a compliant artery produces a reduced $\Delta p$ at similar flow rates [66]. In addition, for the present study, the hyperemic flow rates for serial stenoses were estimated using a CFR-$p_{th}$ plot obtained for patients undergoing PTCA of a single coronary stenosis. In the presence of serial stenoses, the CFR-$p_{th}$ line may have a somewhat reduced slope and this may cause some added reduction in $Qh$. However, we do not expect such a small reduction in $Qh$ to alter the diagnostic parameters observed in this study. Additionally, the close match of our results with the clinical data lends credibility to our assumption.

4.2 Limitations and Future Work. The individual stenoses placed in series were assumed to have a concentric shape with fixed geometric parameters such as the percentage of area blockage, converging and diverging angles, and length of stenosis [43]. Although the heterogeneity in these geometric parameters, observed in a clinical scenario, could produce variations in the results, it is the % AS that has the primary influence on the pressure drop data [67,68]. Thus, the length of an individual stenosis, compared to the % AS, has a lesser effect on $Q$ and $\Delta p$ due to the lesser change in viscous loss compared to the loss due to a change in momentum. Furthermore, all of the combinations of serial stenoses were tested under a fixed connecting length, which allowed for the reattachment of flow distal to the upstream stenosis. Thus, for the current study, the connecting length is sufficient for the flow to be fully developed in the downstream stenosis. However, the reduction in length separating the two stenoses may influence the pressure drop across the stenoses’ combination. Therefore, the effect of the variation in length between the two stenoses on the diagnostic parameters, the FFR and CDP, needs to be assessed in the future. In addition, the stenosis length and % AS need to be studied further in combination.

The experiments were conducted with an antegrade flow across the stenoses; thus neglecting the effect of branched or collateral flow between the stenoses. Such flow may influence the pressures distal and proximal to the upstream and downstream stenosis, respectively, and hence affect the total pressure drop [21]. Therefore, future studies on serial stenoses that account for the presence of collaterals and branched flow are needed.

5 Conclusion

In the presence of serial coronary stenoses, the hyperemic flow $Qh$ significantly reduced with an increasing severity of either the upstream stenosis or the downstream stenosis. Such a decrease in $Qh$ led to the decrease in the pressure drop $\Delta p$ across either of the serial stenosis. Consequently, when the downstream stenosis severity increased from mild to severe, the FFR of the upstream mild, intermediate, and severe stenosis increased by a maximum of 3%, 13%, and 19%, respectively. For an upstream intermediate stenosis, the FFR increased and was either within or above the “gray” range of 0.75–0.80. Therefore, this increase in the FFR above the clinical cut-off would lead to an incorrect deferral of clinical intervention. This may cause misinterpretation of stenosis severity. Considering the wide range of CDP, the CDP of the upstream stenosis varied insignificantly in the presence of a downstream stenosis with a varying degree of severity. However, a distinct range of CDP values for each case of upstream stenosis (mild: 8–10; intermediate: 47–54; and severe: 130–155) allowed for better diagnosis of the upstream stenosis severity in the presence of a downstream stenosis.

Thus, the complex hemodynamic interactions between serial stenoses may affect the hyperemic flow, pressure drop, and FFR across the individual stenosis when placed in series. For the previously discussed scenarios, the FFR may fail to account for the severity of the individual stenosis while assessing serial stenoses. In contrast, CDP, which combines pressure and flow, may better delineate the effect of serial stenoses; thus, holding the promise...
for better functional assessment and clinical diagnosis of individual stenosis severity.

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Appendix
The hyperemic flow, \( \tilde{Q}_h \), and upstream pressure drop, \( \Delta P_u \), evaluated under condition C2 (guidewire across the upstream stenosis only), decreased when the downstream stenosis severity was increased (see Fig. 7). These values are comparable to those obtained under condition C1 (guidewire across both stenoses) (see Table 2). The FFR of the upstream mild, intermediate, and severe stenosis, evaluated under condition C2, increased by a maximum of 3%, 12%, and 16%, respectively, when the downstream severity increased from a mild to a severe condition (see Table 3). With the guidewire pull-back across the downstream stenosis, the FFR of the clinically relevant upstream intermediate stenosis was overestimated (0.86; see Fig. 8(a)) above the clinical cut-off, thus leading to the possible misinterpretation of stenosis severity. Thus, similar trends in the FFR were observed under conditions C1 and C2, except for the 90%–90% AS case, where the FFR of the severe upstream stenosis was below the “gray” zone when evaluated under the C2 condition (see Fig. 8(a)). Thus, the diagnostic misinterpretation may not occur for this scenario. CDP, evaluated under condition C2 (see Fig. 8(b)), showed a distinct range of values (mild: 7–13; intermediate: 47–52; and severe: 130–155) for each case of upstream stenosis in the presence of a varying degree of downstream stenosis. Hence, the nonoverlapping range of the CDP may better delineate the effect of the downstream stenosis from the upstream stenosis.

References


