Focus on flow: imaging the human microcirculation in perioperative and intensive care medicine
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Citation for published version (APA):
Elbers, P. W. G. (2010). Focus on flow: imaging the human microcirculation in perioperative and intensive care medicine
Chapter 9

Direct Observation of the Human Microcirculation during Cardiopulmonary Bypass: Effects of Pulsatile Perfusion
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Journal of Cardiothoracic and Vascular Anesthesia, 2010; epub ahead of print
Abstract

Objectives: Possible benefits of pulsatile perfusion during cardiopulmonary bypass are often attributed to enhanced microvascular flow. However, there is no evidence to support this in humans. Therefore, we assessed whether pulsatile perfusion alters human microvascular flow.

Design: A prospective, randomized observational crossover study.

Setting: A tertiary cardiothoracic surgery referral center.

Participants: Sixteen patients undergoing routine cardiopulmonary bypass for cardiac surgery.

Interventions: All patients underwent both pulsatile and nonpulsatile perfusion in random order.

Measurements and Main Results: We used sidestream dark field imaging to record video clips of the sublingual human microcirculation. Perfusion was started either in the pulsatile (n=8) or the nonpulsatile mode. After 10 minutes, microvascular recordings were made. The perfusion mode was then switched, and after 10 minutes, new microvascular recordings were taken. We quantified pulsatile perfusion-generated surplus hemodynamic energy by calculating pulse pressure and energy-equivalent pressure. Microvascular analysis included determination of the perfused vessel density (mean (standard deviation)). This did not differ between nonpulsatile and pulsatile perfusion (6.65 (1.39) v 6.83 (1.23) mm⁻¹, p=0.58, and 2.16 (0.64) v 1.96 (0.48) mm⁻¹, p=0.20 for small and large microvessels, respectively, cutoff diameter 20 µm). Pulse pressure and energy-equivalent pressure was higher during pulsatile perfusion. However, there was no correlation between the difference in energy-equivalent pressure or pulse pressure and perfused vessel density (r =-0.43, p =0.13, and r=-0.09, p=0.76, respectively).

Conclusions: Pulsatile perfusion does not alter human microvascular perfusion using standard equipment in routine cardiac surgery. Changes in pulse pressure or energy-equivalent pressure bear no obvious relationship with microcirculatory parameters.
Introduction

The possible benefits of pulsatile perfusion (PP) over non-pulsatile perfusion (NP) during cardiopulmonary bypass (CPB) for cardiac surgery remain heavily debated. Some studies have suggested that PP induces hemodynamic and metabolic benefit with improved organ function, but conflicting findings have also been reported [1, 2]. Indeed a recent review states that no recommendation for PP over NP or vice versa can be made when focussing on mortality and incidence of myocardial infarction, stroke, or renal failure [2].

There may be at least two reasons why some studies fail to show benefits of PP. First, the goal of PP is to increase hemodynamic energy. It has been proposed that a certain minimum PP induced surplus hemodynamic energy (SHE) is necessary to unveil its benefits [2]. Negative PP trials may have been conducted using too little SHE. However, the magnitude of a SHE minimum is not known and there is considerable discussion on how it should be measured [2].

Second, the benefits of PP may remain invisible amidst many other clinical factors influencing these commonly reported outcomes. In addition, the incidence of these adverse events in routine cardiac surgery is already very low, necessitating very large trials for a beneficial effect to become apparent. This implies that routinely applying PP using standard CPB equipment may lack rationale because SHE may be insufficient or its effects are too marginal.

Mechanistically, PP is thought to improve microvascular flow. This is consistent with the concept that the microcirculation is crucial for oxygen and nutrient delivery to tissue and ultimately for organ function [3]. As reviewed by Ji and Undar, evidence for microvascular improvement is available but scarce and mainly based on surrogate markers such as laser Doppler flux and ultrasonic flow probes [4]. While valuable, these techniques cannot distinguish capillary from larger vessel flow.

Until recently, the lack of suitable techniques has limited direct microscopic observation of the microcirculation to animal studies. This has provided evidence of improved capillary flow in rats and goats [4]. However, with the recent advent of side stream dark field (SDF) imaging it has now become possible to observe the human microcirculation in real time [5]. An intriguing and consistent finding using this technique in a variety of clinical settings has been that microvascular flow is relatively independent from global hemodynamics.

Against this background, it is relevant to assess whether PP generated using standard CPB equipment does actually alter microvascular perfusion. Therefore we used SDF imaging to record video clips of the sublingual microcirculation in patients undergoing cardiac surgery using commonly available CPB equipment. Using a crossover design, this enabled us to assess their capillary perfusion during both PP and NP. It was our hypothesis that indices of microvascular perfusion would improve using PP as compared to NP.
Methods

This study was approved by our local institutional review board. The need for written informed consent was waived in accordance with the national Law on Experiments with Humans because routine procedures and equipment were used and microvascular measurements were considered non-invasive.

We studied adult patients undergoing coronary artery bypass grafting or aortic valve replacement necessitating CPB. Lacerations of the oral mucosa were a criterion for exclusion because of interference with microcirculatory imaging. Another exclusion criterion was bolus injection of vasopressors. Routine monitoring of global hemodynamics included invasive arterial and central venous blood pressures.

General procedure

Patients received fentanyl, pancuronium, propofol and remifentanil for anesthesia induction and maintenance. No volatile anesthetics were given. After midline sternotomy and systemic heparinization, a 24 F cannula and a 36/51 F two-stage cannula (Medtronic, Heerlen, The Netherlands) was used for aortic and right atrial cannulation, respectively. Cardiopulmonary bypass was initiated using a Jostra-HL30 (Maquet, Hilversum, The Netherlands) heart-lung machine with a RP150 arterial roller pump, Quadrox membrane oxygenator, and Maquet custom pack tubing. A Veri-Q Doppler flow meter (Medistim, Deisenhofen, Germany) and pressure transducer were connected to the arterial side of the CPB circuit, distal to the oxygenator. For cardioplegia, 800-1000 mL cold low sodium crystalloid cardioplegic solution was administered. Flow rate during CPB was aimed at 2.4 L/min/m² at body temperature of 32 °C using either PP or NP (see below).

Experimental procedure

Patients were randomly assigned to start on either PP or NP by opening sealed envelopes after patients were included. For PP we used pulsatility settings with a baseline flow of 35% of the average flow and a pulse starting at 55% and stopping at 80% of the 65 min⁻¹ cycle. This is our routine setting producing a maximum pulse while preventing negative oxygenator pressures.

Ten minutes after administration of cardioplegic solution, we obtained microvascular video recordings of the sublingual microcirculation (see below). We chose the sublingual area for its phylogenetic relation to the gut and ease of access. Fifteen minutes after administration of cardioplegic solution, CPB mode was switched, i.e. patients that started on PP were put on NP and vice versa. Ten minutes later, we obtained a second series of sublingual microvascular video recordings.

Microvascular recordings

We used SDF imaging to obtain microvascular recordings. This technique has been de-
scribed in detail previously [5]. In brief, it consists of a handheld video microscope that emits stroboscopic green light (wavelength 530 nm) from a probe that is absorbed by hemoglobin. Thus, a negative image of moving red blood cells is transmitted back through the isolated optical core of the probe towards a charge-coupled device camera. SDF imaging has been shown to provide a higher image quality with more detail and less motion blur than its predecessor OPS imaging [5].

Recordings were performed in accordance with recommendations from a recent round table conference [6]. Video recordings yielding at least 20 second of stable images were digitally stored and represent approximately 940x750 µm$^2$ of tissue surface. For each time point, SDF recordings at 3 different sublingual sites were recorded within five minutes. Special care was taken to avoid pressure artifacts [6, 7].

**Microvascular analysis**

Evaluation of microvascular recordings was in accordance with recommendations from a recent round table conference [6]. All images were given a random number and analyzed offline by one of the authors (PWGE). We determined perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI) and indices of heterogeneity [8-11]. For PPV and PVD, vessel density was calculated as the number of vessels crossing 3 horizontal and 3 vertical equidistant lines spanning the screen divided by the total length of the lines. Perfusion at each crossing was then scored semi-quantitatively as follows: 0 = no flow (no flow present for the entire duration of the recording), 1 = intermittent flow (flow present <50% of the duration of the recording), 2 = sluggish flow (flow present >50% but <100% of the duration of the recording or very slow flow for the entire duration of the clip), and 3 = continuous flow (flow present for the entire duration of the recording). PVD was then calculated as the number of crossings with flow scores greater than 1. PPV was calculated as the proportion of crossings with flow scores greater than 1 divided by the total number of crossings. For each time point and each patient, the scores for PPV and PVD were averaged.

PPV is expressed as n/mm, whereas PVD is expressed as a percentage. MFI was based on the determination of the predominant type of flow in 4 quadrants adhering to the same
scoring system. MFI is the sum of these flow scores divided by the number of quadrants in which the vessel type is visible. Heterogeneity was assessed in 2 different ways. For PVD, the coefficient of variation was determined [7]. For MFI, we assessed heterogeneity in each patient by subtracting the lowest from the highest quadrant MFI and dividing the result by the mean MFI [11].

To determine intra- and interrater variability, 17 randomly chosen video recordings were analyzed again after 10 weeks independently by the same and a different observer. For PVD, for all vessel sizes, Bland’s intrarater bias was between -0.03 (0.28) and 0.28 (0.47) mm\(^{-1}\) with an absolute mean difference of 0.17-0.41, which is 5.6%-9.3% of mean PVD [12]. Interrater bias was between –0.03 (0.28) and 0.64 (0.82) mm\(^{-1}\) with an absolute mean difference of 0.17-0.78 mm\(^{-1}\) which is 7.3–10.1% of mean PVD. De Backer et al., studying microvascular blood flow in septic patients, previously reported intrarater variability ranging from 2.5%-4.7% and interrater variability ranging from 3.0-6.2% (8). For PPV, for all vessel sizes, intrarater bias was between 0 (0) and 0.66 (2.23) % with an absolute mean difference 0-1.35%, which is 0–1.41% of mean PPV. Inter-rater bias was between 0 and -1.34 (2.11) % with an absolute mean difference of 0 to 1.64% which is 0 to 1.7% of mean

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) or n (%)</th>
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<tbody>
<tr>
<td>Male</td>
<td>8 (50%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.6 (11.7)</td>
</tr>
<tr>
<td>CABG</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>AVR</td>
<td>8 (50%)</td>
</tr>
<tr>
<td>Morrow</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Re-operation</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Beta blocker use</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>ACEI/ARA use</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Calcium antagonist use</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Propofol (mg/h)</td>
<td>241 (58)</td>
</tr>
<tr>
<td>Remifentanil (mg/h)</td>
<td>0.60 (0.55)</td>
</tr>
<tr>
<td>Nitroglycerin (mg/h)</td>
<td>0.56 (0.51)</td>
</tr>
</tbody>
</table>

Table 1. Patient characteristics. ACEI/ARA, angiotensin converting enzyme inhibitor/angiotensin receptor antagonist; AVR, aortic valve replacement; CABG, coronary artery bypass grafting; Morrow – morrow procedure, i.e. ventricular septal myotomy/myectomy.
De Backer reported intra- and interobserver variability for PPV to be between 0.9-4.5% and 4.1-10%, respectively [8]. For MFI, intraobserver kappa score was between 0.903 and 0.954 [13]. Boerma et al. and Trzeciak et al. previously performed intra- and interrater variability in septic patients. Boerma et al. found an intraobserver kappa score of 0.78 [11]. Interobserver kappa score for MFI was between 0.821 and 1 whereas Boerma and Trzeciak reported values of 0.85 and 0.77 [7, 11].

**Quantification of hemodynamic energy**

Pulse pressure was calculated as the difference between systolic and diastolic radial artery pressure. We continuously and simultaneously monitored pressure waveforms just proximal to the aortic cannula and flow waveforms just distal to the oxygenator to quantify CPB circuit Energy Equivalent Pressure (EEP). This was calculated using Shepard’s formula to quantify the pressure and flow waveforms in terms of hemodynamic energy. It is based on the ratio between the area beneath the hemodynamic power curve (integral fpdt) and the area beneath the pump flow curve (integral fdt) during each pulse cycle (14): EEP = (integral fpdt)/(integral fdt). Where f is the pump flow rate, p is the arterial pressure (mm Hg), and dt indicates that the integration is performed over time (t). The unit of the EEP is mm Hg. The difference between the EEP and MAP represents the surplus hemodynamic energy (SHE) generated by each pulsatile or non-pulsatile device.

**Statistics**

We used paired t tests to analyse all data except for MFI for which we used Wilcoxon signed rank tests. Results are reported as median and interquartile ranges (IQR) for MFI and as the mean (SD) for other parameters. Spearman tests were used to detect possible correla-
tion between small vessel PVD and both EEP and pulse pressure. Power calculation was performed before the start of the study. We calculated that 14 patients would be required to detect a 15% difference in PVD between NP and PP based on previous studies by us and others [8, 15, 16]. In order to account for possible data loss, we planned to include 16 patients.

**Results**

We included 16 patients. Half of these started CPB in PP mode. Figure 1 shows a typical flow recording of both PP and NP modes. Patient characteristics including details on surgery are listed in table 1. One patient received noradrenaline during imaging and was excluded. In all other patients, no dose adjustments of anesthetic and/or vasoactive drugs were made between measurements. One patient was excluded from hemodynamic energy analysis because of technical failure in pressure and flow recordings.

Table 2 lists the hemodynamic results. There was no difference in CPB flow rate or mean arterial pressure (MAP) between groups. As intended, pulse pressure and EEP was signifi-
significantly higher for PP. A still of a typical microvascular video recording is shown in figure 2. Results of microvascular analysis may be found in Figure 3 and Table 3. There was no difference in any microvascular parameter between NP and PP. This is true both for smaller and larger microvessels. Similarly, indices of microvascular heterogeneity did not differ between groups for either vessel size. Figures 4 and 5 plot the difference in small vessel PVD against the difference in pulse pressure and EEP. No significant correlation could be found for either (Spearman $r=-0.43$, $p=0.13$ for EEP and Spearman $r=-0.09$, $p=0.76$ for pulse pressure).

**Discussion**

This is the first study to report on the microvascular response to PP during routine cardiac surgery using standard CPB equipment. The most important result is that PP does not alter microvascular perfusion as compared to NP in this setting in the face of a significant rise

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>PP</th>
<th>Difference and 95%-CID</th>
<th>p</th>
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<tbody>
<tr>
<td>Ø &lt; 20 µm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVD</td>
<td>6.65 (1.39)</td>
<td>6.83 (1.23)</td>
<td>0.18 (-0.51 to 0.87)</td>
<td>0.58</td>
</tr>
<tr>
<td>PPV</td>
<td>94.7 (5.0)</td>
<td>92.8 (8.4)</td>
<td>-1.9 (-4.9 to 1.1)</td>
<td>0.20</td>
</tr>
<tr>
<td>HI-PVD</td>
<td>0.22 (0.14)</td>
<td>0.18 (0.15)</td>
<td>-0.04 (-0.12 to 0.04)</td>
<td>0.33</td>
</tr>
<tr>
<td>MFI</td>
<td>3.00 (2.83 to 3.00)</td>
<td>3.00 (2.75 to 3.00)</td>
<td>n/a</td>
<td>0.41</td>
</tr>
<tr>
<td>HI-MFI</td>
<td>0.25 (0.35)</td>
<td>0.33 (0.46)</td>
<td>0.08 (-0.15 to 0.32)</td>
<td>0.46</td>
</tr>
<tr>
<td>Ø &lt; 20 µm</td>
<td></td>
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</tr>
<tr>
<td>PVD</td>
<td>2.16 (0.64)</td>
<td>1.96 (0.48)</td>
<td>-0.20 (-0.50 to 0.11)</td>
<td>0.20</td>
</tr>
<tr>
<td>PPV</td>
<td>99.8 (0.7)</td>
<td>99.1 (3.7)</td>
<td>-1.7 (-3.8 to 0.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>HI-PVD</td>
<td>0.28 (0.15)</td>
<td>0.40 (0.25)</td>
<td>0.12 (-0.04 to 0.28)</td>
<td>0.12</td>
</tr>
<tr>
<td>MFI</td>
<td>3.00 (2.75-3.00)</td>
<td>3.00 (3.00 to 3.00)</td>
<td>n/a</td>
<td>0.50</td>
</tr>
<tr>
<td>HI-MFI</td>
<td>0.05 (0.12)</td>
<td>0.12 (0.27)</td>
<td>0.08 (-0.10 to 0.26)</td>
<td>0.36</td>
</tr>
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Table 3. Microvascular parameters. NP – non-pulsatile perfusion; PP – pulsatile perfusion; Δ and 95%-CID – difference and 95% confidence interval of the difference; PVD – perfused vessel density; PPV – proportion of perfused vessels; HI – heterogeneity index; MFI – microvascular flow index; n/a – not applicable.
in both EEP (and thus SHE) and pulse pressure. This adds to the large body of evidence that macrovascular hemodynamic parameters do not necessarily reflect microvascular perfusion. This implies that global hemodynamics and microvascular flow are dissociated in this setting. However it must be noted that our results pertain only to the sublingual microvascular bed at a single level of pulsatility.

Multiple mechanisms have been postulated for a possible PP induced beneficial microvascular effect. Among these are recruitment and prevention of derecruitment of capillaries, prevention of capillary sludging, and promotion of lymph movement [17, 18]. While the latter cannot be visualized using SDF imaging, capillary recruitment and sludging can readily be seen and quantified. This would be consistent with a higher PVD and PPV and lower indices of heterogeneity when using PP. However, as stated above, no differences in these parameters were found.

With SDF imaging, arterioles are usually not visible and the images usually consist of capillaries and venules only. No pulsatility in any vessel type in any patient in any perfusion mode could be confirmed during direct visualization. We did measure heterogeneity, but found no change during PP compared to NP. This could mean that even in patients with large EEP differences, the surplus energy is still insufficient to propel pulsatility beyond the arterioles.

Figure 3. Perfused Vessel Density for small microvessels (Ø <20 μm) during pulsatile (PP) and non-pulsatile (NP) perfusion. Each dot represents one patient.
An alternative explanation would be that pulsatility cannot be achieved in these downstream microvascular vessels, regardless of the amount of surplus energy. Instead, this energy might ultimately be used to open up previously closed capillaries. Indeed, this was the case in the goat studies showing an almost a twofold increase in PVD following PP [17, 19-21]. However, these changes could not be reproduced in a goat having only a left ventricular assist device [19]. Also using intravital microscopy in a goat model, a Canadian study by Lee et al could not show a difference in perfused capillary density in the flexor carpi ulnaris muscle using PP [22].

There may be several possible explanations for the fact that we failed to observe microvascular improvement using PP whereas several animal studies did. First, aortic cannulas are known to induce energy loss both in general and for pulsatility added energy specifically [23]. Similarly, Undar et al. have clearly shown that different pumps producing the same pulse pressure may have very different hemodynamic energy levels [24]. Unfortunately we only measured EEP in the CPB circuit. This implies that the absence of microvascular alterations in our study may have been caused by a failure to transmit SHE to the arterial

Figure 4. Plotted is the difference in pulse pressure between pulsatile and non-pulsatile perfusion versus the corresponding difference in perfused vessel density for small microvessels ($\Omega < 20 \mu m$). There does not seem to be an obvious relation between these parameters.
system. However, as clearly shown in figures 4 and 5, there is no correlation between the change in pulse pressure or EEP and microvascular perfusion. This may support the view that even if SHE is properly transmitted microvascular alterations may still not emerge. Voss et al recently submitted pigs to near physiological PP by a custom made device and found no difference in renal and intestinal blood flow [25]. This is a finding that probably points in the same direction. Second, our patients underwent PP and NP for a relatively short time. Perhaps a difference in microcirculatory indices would have emerged if PP would have been applied longer. Finally, possible PP benefits may only become apparent in fragile patients, especially those with disseminated atherosclerotic pathology [2, 4]. Perhaps our routine patients were too healthy to show any PP induced microvascular improvement, although we did not quantify their chronic health. In fact, all PVD values are similar to those in routine patients after cardiac surgery in our ICU [16].

It was our specific goals to assess the microvascular effects of PP using a readily available heart lung machine. Interestingly our type of heart lung machine and oxygenator is known to provide more hemodynamic energy than others [24, 26]. Therefore, although speculative, it seems likely that other commercially available setups will similarly fail to improve

Figure 5. Plotted is the difference in energy equivalent pressure at the level of the extracorporeal circuit (EEPpump) between pulsatile and non-pulsatile perfusion versus the corresponding difference in perfused vessel density for small microvessels (Ø <20 µm). There does not seem to be an obvious relation between these parameters.
We recognize that our study has some important limitations. First, we chose the sublingual area for microvascular imaging for its ease of access, its location in the core temperature shell, its phylogenetic relation to the gut and its proximity to the brain. However the sublingual microcirculation does not necessarily reflect other microvascular beds [27, 28]. Further, the technique of SDF imaging carries a risk of operator induced movement bias. However, by strictly applying every recommendation given in the round table conference, we believe this risk was minimal. Finally, it is known that initiating CPB per se may compromise microvascular flow though this is mild and restored towards normal during the course of CPB [29, 30]. However, we circumvented this potential source of bias by starting with NP in half of our patients while starting with PP in the other half.

In summary, we studied microvascular effects of PP in a clinically relevant setting of cardiac surgery. Despite a significant PP induced increase in pump generated hemodynamic energy and pulse pressure, we found no PP induced improvement in microvascular flow. However it should be remembered that we only imaged the sublingual microcirculation at one level of pulsatility in routine cardiac surgery. Further studies may be needed to characterize the microvascular effects of PP in a more comorbid population.

References


