Multiscale analysis of coronary branching and collateral connectivity: coupling vascular structure and perfusion in 3D
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Chapter 8

Influence of imaging resolution on hyperemic flow prediction from vessel lumen volume

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Abstract

An allometric scaling law between the coronary vascular volume (V) and hyperemic blood flow (Q) is currently investigated for the noninvasive estimation of the functional impact of coronary artery disease. A value of 0.75 for the exponent $\beta$ of the scaling law, $Q=\alpha V^{\beta}$ (with scale factor $\alpha$), is used in in vivo angiograms with a spatial resolution of 500 $\mu$m. Our hypothesis is that inclusion of smaller vessels may lead to a different relationship between V and Q.

We compared the relation between V and Q at increasing levels of truncating the coronary tree. A relative measurement of Q was assessed by injecting fluorescent microspheres into the coronary vessels of a canine heart at maximal vasodilation. The heart was excised and the coronaries were filled with fluorescent cast material. The specimen was cut with an imaging cryomicrotome. Microsphere locations were used to derive the relative Q and vascular volume was determined for increasing truncation, starting from terminal segments at 50 $\mu$m radius up to 1000 $\mu$m.

Crown volumes were increasingly affected by radius truncation for smaller stem segments. The scaling law exponent $\beta$ decreased when the truncation level of included vessel radii was increased. Radius truncation did not affect the factor $\alpha$. A value for exponent $\beta$ was found of 0.79 for 50 $\mu$m radius truncation and 0.55 for 1000 $\mu$m radius truncation.

Cryomicrotome analysis allows simultaneous measurements of flow and vascular structure down to the microvascular level and therefore allows investigation into the scaling law behavior. The scaling law exponent $\beta$ depends on the truncation level and a correct value is paramount for estimation of hyperemic blood flow.
**Introduction**

Fractional Flow Reserve, FFR, is a physiological index used clinically during PTCA procedures to decide for intervention in case of a coronary stenosis. Conceptually, FFR is defined as the hyperemic flow in a vessel with stenosis divided by the hyperemic flow when the stenosis would not be present. Normally, FFR is measured as the ratio between the pressure measured distally of a stenosis during hyperemia induced by adenosine and aortic pressure. Measurement of FFR requires a guide wire with pressure sensor to be passed through the stenosis.

Recently a method has been proposed for measuring FFR without a pressure sensor equipped guide wire. Impeded flow in the presence of a stenosis is measured by the wash in of angiographic contrast medium [1] and the hyperemic flow in the absence of the stenosis is predicted from the volume of the vascular tree distal of the stenosis [2]. Hence, it is assumed that a fixed relation between these two quantities under hyperemic conditions exists.

The relationship between hyperemic flow and arterial vascular volume is derived from allometric scaling laws of various anatomical properties of the vascular tree [3]. This scaling law principle can be applied to a subtree level and hence between any segment of the tree and its corresponding distal vascular bed. From these allometric relations, one ultimately arrives at a scaling law relation between total lumen volume \( V \) and hyperemic flow \( Q \), of the form \( Q = aV^\beta \) [4], with exponent \( \beta \), and factor \( a \).

The relation between \( V \) and \( Q \) strongly depends on the value of \( \beta \). A value for the exponent of \( 3/4 \) (0.75), was previously derived based on the measured relationship between flow and segment length [3] and the derived relationship between segment length and crown volume [3]. This value was validated by angiographic imaging limited by a resolution of 500 \( \mu \)m [2, 5-7]. Solely by analytical derivation, a value of 7/9 (0.78) has been suggested recently [8]. Flow simulations with coronary artery tree models derived from morphometric properties determined from corrosion cast studies resulted in predicted values in the range of 0.7 to 0.77. These models demonstrated an effect of truncation radius when varied between 8 \( \mu \)m and 500 \( \mu \)m on \( \beta \) [9].

The impact of exclusion of vascular volume by vessel truncation was investigated for scaling law behavior between strictly anatomical variables, such as vascular volume and cumulative branch length [10] and showed no significant difference in exponent for increased radius truncation. However, anatomical variables would equally be affected by this radius truncation. The effect of radius truncation was not directly investigated on the \( Q-V \) scaling law behavior.

This study aimed at analyzing the scaling law relationship of the coronary arterial tree between flow in a segment and its crown volume using the imaging cryomicrotome [11]. Flow distribution has been measured by microsphere injections and crown volume from 3D reconstruction of vascular bed filled with fluorescent replica material. We hypothesized that there is a difference in scaling law dependent on the diameter range of vessels included in the volume measurements.
Material and methods

Canine datasets

Five canine heart datasets for this study were obtained from a series of physiological experiments carried out according to a protocol that was approved by the Institutional Animal Care and Use Committee of the University of Utrecht Medical Center. Before removal of the hearts, microspheres (Invitrogen, molecular probes) labeled with carmine fluorescent (582ex/614em) were injected into the Left Anterior Descending artery (LAD) at maximal hyperemia, while a flow probe (Transonic systems, USA) proximal to the injection site of the LAD, measured flow during injection. A single batch of a fluorescent color contained approximately 100,000 microspheres. After the experiment, the animal was sacrificed by fibrillating the heart after which it was excised and the right and left main coronary arteries were cannulated and perfused with a buffered solution containing adenosine for vasodilation of the microcirculation. For analysis of the vascular tree, the coronary arteries were filled at physiological pressure with a fluorescent cast material (Batson no. 17, Polysciences, USA) consisting of a monomer base solution, a catalyst and a promoter as described previously [11]. Potomac Yellow (440ex/490em) (Radiant Colour, Belgium) provided the fluorescent base for the replica plastic. The cast material was allowed to harden over a period of 24 hours at ambient temperature. The fully prepared heart was embedded in carboxymethylcellulose sodium solvent 5% (Brunschwig Chemie, The Netherlands) and Indian ink 5% (Royal Talens, The Netherlands), frozen at -20°C and prepared for cryomicrotome analysis.

The imaging cryomicrotome

The cryomicrotome setup and imaging procedure was described in detail by Spaan et al. [11]. Some modifications were implemented since then and used in the present procedure. In brief, the frozen heart was mounted with its long axis perpendicular to the cutting plane, which was illuminated with a cluster of 7 power light emitting diodes (LED) (Luxeon V, Star, Royal Blue, Lumileds Lighting, United States). The specimen was cut, from base to apex, at 25 µm slice thickness using a motorized automated cutting mechanism. After each cut, the block face of the remaining bulk material was imaged using a 4096x4096 pixel CCD camera (Apogee Alta U-16, USA) equipped with a variable focus lens (Nikon 70-180mm, The Netherlands). For each cut, a black and white photo of the of the heart and an emission image of the respective fluorescent cast material and fluorescent microspheres were obtained after optical filtering at 505 nm or 635 nm. Sequential images with an in-plane resolution of 25 µm pixel were stored to yield a registered image stack representation the 3D virtual morphology of the detailed vascular network, microsphere locations and surrounding tissue.

Image restoration

Image degradation due to scattering of emission light in the tissue, lens blurring and light coming from structures beneath the imaged surface was corrected for by deconvolution with a system-specific point spread function (PSF) [12]. The correction of the image stack was
performed in the spatial domain using a custom-made 3D iterative deconvolution program written in CUDA (Nvidia, USA).

Deconvolution with the PSF on microsphere data would result into microspheres being reduced to single pixels and made indistinguishable from background noise. The microsphere data was therefore restored by doing a per slice removal of the transparency artifacts followed by microsphere detection [13].

**Vascular tree segmentation and quantification**

After deconvolution, the vessel outlines were skeletonized using a topology-preserving thinning algorithm [14]. Points on the centerlines were classified according to their nearest neighbor connections as endpoints, with one neighbor; midpoints, with two neighbors; and bifurcations, in case of three neighbors. Higher orders of connectivity due to thinning artifacts were replaced by bifurcations. Vessel segments were defined between consecutive non-midpoints. The segmented tree represented the 3D topological vascular network.

Local diameters were determined by examining the cross-sectional intensity profile along 64 radial vectors perpendicular to the segment centerline for each mid-point. With the vessel center as the maximum intensity, the full width at half maximum intensity along each vector was found and averaged to yield the mid-point diameter. The cross-section of the segment was assumed to be circular with constant diameter defined by averaging over its mid-point diameters.

**Scaling law Q-V determination**

The coronary arterial system was reconstructed in 3D down to the smallest observable segment with radius of 25 µm. Subsequently, each detected microsphere was mapped to the segment with the shortest distance to its centerline. The relationship between segmental flow and crown volume was determined as illustrated in Fig. 1.

![Fig. 1](image-url): Schematic representation of a subtree with stem segment k and corresponding crown volume $V_k$. Microspheres within the contour are mapped to the distal vascular tree as a relative measurement of perfusion, $M_k$, that passed through the stem segment.
All five datasets were limited to vessels with a lower threshold of 50 µm radius, to ensure a uniform terminal segment radius. The volume of each individual segment, $v_i$, was determined from its estimated diameter, $d_i$, and length $L_i$ by assuming a cylindrical shape. For a chosen segment $k$, denoted as stem, all $N^k$ downstream segments are identified and summed to obtain the corresponding crown volume $V_k$. Hence the total vascular crown volume is:

$$V_k = \sum_{i=1}^{N^k} v_i$$  \hspace{1cm} (1)

The total number of microspheres that passed through the stem can be determined from the sum of all microspheres mapped to the segments of the downstream tree indexed by $j$:

$$M_k = \sum_{j=1}^{N^k} m_j$$  \hspace{1cm} (2)

The number of microspheres that passed through a segment $k$ is proportional to flow by:

$$Q_k = k_q M_k$$  \hspace{1cm} (3)

with $k_q$, the factor relating number of microspheres to flow, obtained by direct flow measurements during microsphere injection. The scaling law expression between flow and cumulative volume then results into;

$$Q_k = \alpha V_k^\beta$$  \hspace{1cm} (4)

The volume and flow data was used to obtain the value of exponent $\beta$ and factor $\alpha$.

**Vessel truncation**

Vessel truncation was done by excluding vasculature below a threshold radius from the volume measurements, demonstrated in Fig. 2. Truncation was done in steps of 100 µm radius starting from 100 µm to 1000 µm. A crown volume obtained with threshold at radius $r$ is referred to as $V_r$. The influence of vessel truncation was always investigated for stem segments and their corresponding crown volume of 1000 µm radius and larger. This corresponds to the typical vessel size that is evaluated for stenosis severity in a clinical setting.
Fig. 2: A) Raw vascular cast data obtained after cryomicrotome analysis of the full tree. B) Segmented representation of the raw data down to 50 µm radius vessels. C) Representation of the dataset for 200 µm radius truncation.

**Error in flow prediction**

According to Eq. 4, hyperemic flow can be predicted from crown volume at given β. We take β derived from truncating the crown at vessels of 500 µm as reference. Hyperemic flow can then be calculated for an arbitrary truncation values indicated as βr. The relative difference of flow with reference prediction can be written as equation 5 for α is constant:

\[
Q_{\text{error}}(\%) = \left( \frac{V_{\beta_{500}}}{V_{\beta_r}} - 1 \right) \times 100
\]  

(5)

This effectively represents the clinical setting were a fixed scaling law is used, even though the imaging resolution would require an adaptation to the exponent value. The error estimate was investigated for the volume range from 0 ml to 1.2 ml containing the physiologic range.

This thresholding obviously has also an effect on the calculation of FFR. The induced error was analyzed for FFR prediction of 0.75. For the same baseline flow, FFR alters with the hyperemic flow as function of βr as defined by equation 5. FFR obtained from the reference β500 with Qs as impeded flow through the stenosis can be written as:

\[
F FR_{\text{ref}} = 0.75 = \frac{Q_s}{Q(\beta_{500})}
\]

(6)

The FFR value estimated as function of βr then becomes:

\[
F FR = \frac{Q_s}{Q(\beta_r)}
\]

(7)

Error estimate in FFR prediction compared to the reference FFR of 0.75 can then be written as, by substituting Eq. 6 into Eq. 7:

\[
F FR = 0.75 \times \frac{Q(\beta_{500})}{Q(\beta_r)}
\]

(8)
Statistical methods

A linear regression fit was applied to the log of the vessel volume and corresponding log of the flow data to obtain the values for exponent $\beta$ and factor $\alpha$. Normally distributed data were compared using an paired Students t-test as appropriate. A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed in Graphpad Prism (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Impact of radius truncation on total vessel volume

The impact of vessel truncation for the volume of the coronary vasculature as a whole is demonstrated in Fig. 3. The volume of the tree truncated at 50 $\mu$m radius vessels, was taken as a reference. For different truncation radii the vascular volume was then presented as the fraction of the reference volume. About 75% of the total arterial volume remained after truncation at a radius of 500 $\mu$m. The cumulative volume of the vessels with radius between 50 $\mu$m and 500 $\mu$m therefore contains around 25% of the arterial total volume. For 1000 $\mu$m radius truncation, around 59% of the total arterial volume remained.

![](image)

Fig. 3: Total vascular volume fraction for increased truncation radius for all hearts. The ratio between the volume at 500 $\mu$m truncation ($V_{500}$) and 50 $\mu$m truncation ($V_{50}$) was on average 0.79 and between 1000 $\mu$m truncation ($V_{1000}$) and 50 $\mu$m truncation ($V_{50}$) on average 0.62. There is a mild exponential increase in total vascular volume fraction towards the smaller vasculature.

Impact of radius truncation on stem dependent crown volume

Crown volume is not only related to truncation at defined distal segmental diameter but also to the stem diameter. A more proximal stem will have a larger crown than a more distal stem. The effect of stem diameters on crown volume was analyzed for the truncation thresholds 50 and 500 $\mu$m. In Fig. 4A, the relation between these two crown volumes is
demonstrated for stem segments of the LAD with radius of 1000 μm and larger. Each data point is calculated for a different stem. Fig 4A demonstrates an increase in relative difference between the two volumes with deceasing stem radius.

**Fig 4:** A) Log-log plot of the crown volumes with 50 and 500 μm as terminal vessels belonging to the same stem. Data points are from all stem segments larger than 1000 μm obtained for the same coronary tree. The relationship between the crown volumes is increasingly affected at smaller diameters of the stem segment. The two ranges for smaller and larger stem segments are subdivided by the intersection of the red lines. B) Log hyperemic flow (Q) versus Log volume (V) for three different truncation radii: 50 μm (black dots), 500 μm (grey crosses) and 1000 μm (light grey triangles). Log-log fits were: \( Y = 0.79 \times X + 1.75 \) (\( R^2 = 0.97 \)), \( Y = 0.71 \times X + 1.79 \) (\( R^2 = 0.96 \)) and \( Y = 0.55 \times X + 1.76 \) (\( R^2 = 0.93 \)), for 50 μm, 500 μm and 1000 μm truncation respectively. The slope of the fits becomes less steep for increased vessel radius truncation.

The data in Fig. 4A can be globally subdivided in two ranges around \( \log(V_{50}) = -1.05 \), where in the range for higher volumes and stem segment radii, a power law with exponent 1 can be recognized and for smaller stem diameters with an exponent larger than 1 as indicated in the figure by the intersection of the red lines.

**Volume-flow relationship**

The crown volumes for stem segments larger than 1000 μm and their corresponding flow measurements are plotted in Fig 4B for a typical heart. This is done for 3 different truncation thresholds: \( V_{50} \), \( V_{500} \) and \( V_{1000} \). The Q-V data were fitted by a log-log relation resulting in an exponent value \( \beta \) of 0.79, 0.71 and 0.55 for these three truncation radii respectively. Please note that each stem segment is represented for 3 truncation radii but with essentially the same flow value.
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Fig. 5: A) Exponent value $\beta$ as a function of truncation radius obtained for vessels with a radius greater than 1000 $\mu$m, plotted as mean and standard deviation. A consistent decline is seen towards larger truncation radius. Subsequent values of $\beta$ were significantly smaller at each truncation step ($p<0.05$). B) Similar plot as in A, but now for the factor $\alpha$. No significant change in factor value $\alpha$ was seen for increased radius truncation.

The volume-flow scaling law was evaluated as function of truncation radius for all hearts. Truncation radius was altered from 50 to 100 and with steps of 100 $\mu$m until 1000 $\mu$m. As demonstrated in Fig 5A a non-linear decline in exponent value $\beta$ was observed for increasing truncation radii, $\beta$ was always significantly lower than the value of $\beta$ at a previous truncation step ($p<0.05$). Similarly the factor $\alpha$ was investigated, but showed no significant changes as depicted in Fig 5B. All fits used to obtain $\beta$ and $\alpha$ had a $R^2$ value above 0.7 but was above 0.9 in two thirds of the cases.

**Error estimate of flow and FFR value**

The errors made in hyperemic flow and FFR with the volume based method depends on stem and truncation radius as demonstrated in Fig. 4. However, since crown volume is the clinically measured quantity we analyzed the sensitivity for error in calculating flow and FFR from crown volume in Fig. 6. For this sensitivity analysis, we assume the truncation radius of 500 $\mu$m as the reference as expressed by equation 5. Moreover, literature indicates that crown volume is not larger than 1 ml. Hence for $\beta_{500}$ the error is zero and independent of crown volume. For smaller values of $\beta$ the flow is overestimated and for larger values underestimated.

The estimate in error for FFR is defined by equation 8 and depicted in Fig. 6B for FFR=$0.75$, the earlier clinical threshold for clinical decision making. Again, for $\beta_{500}$ the error is zero per definition. FFR is overestimated for larger and underestimated for smaller truncation radii.

With a crown volume measurement and assuming a truncation radius of 300 $\mu$m, an overestimation can occur ranging from 7% to 3% in flow for a crown volume in the range of 0.2 ml to 0.6 ml. The corresponding predicted FFR values are in range of 0.69 to 0.73, Fig.6B. Larger errors in flow estimates may occur for greater mismatches between the reference $\beta$ and resolution corrected $\beta$, Fig.6A.
Discussion

This is the first time that flow-crown volume relations have been studied experimentally with high resolution of hyperemic flow and vascular volume measurements. These relations confirm scaling law behavior but the exponent depends on the radius of the vessel for which the tree is truncated. The definition of truncation radius can be translated to clinical imaging via the notion resolution. Our results obtained confirm that scaling laws can be applied to estimate hyperemic flow under the conditions that we have measured. However, the exponent of the scaling law has to be adapted to the resolution of the imaging modality.

Data interpretation

In our study the exponent of the scaling law varied from 0.79 to 0.55 with truncation radius varying from 50 to 1000 µm. The lower values agree reasonably with experimental values obtained radiographically at low resolution. The higher value corresponds fairly well with the theoretical value of 0.78 (7/9) derived by Huo and Kassab where they derive the flow-volume relation of the crown based on rules for branching at bifurcations [8]. It should be noted that truncation of the tree does not play a role in this theoretical derivation. It makes sense that our value obtained with the smallest truncation radius fits best to the theoretical value. One may argue that an even smaller truncation radius would have improved the correspondence between theoretical and experimental data. However, our Fig. 3 indicates that volume in the range of vessels smaller than 50 µm can practically be neglected.

The question is whether the correspondence between our experimentally found exponent and the theoretically derived value is accidental. To answer this question one should evaluate the branching rules assumed by Huo and Kassab by our morphological data. We have not done so since the aim was the evaluation of the clinical method to derive FFR from scaling laws. It is not a-priory to be expected that correspondence in exponent implies confirmation

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Fig. 6: A) Error estimate as percentage difference between a reference flow prediction with an exponent value for 500 µm truncation (β_{500}) and a flow predicted at four incrementing levels of radius truncation, β, with r; 50, 300, 600 and 1000 µm. The scaling factor α was assumed constant for different radius truncations. B) Example of an FFR prediction for hyperemic flow estimated with β corresponding to 500 µm radius truncation, on data of levels of radius truncation again for β, with r; 50, 300, 600 and 1000 µm. The actual value of the FFR was chosen as 0.75.
of the assumed branching rules. In an earlier study of the same group an exponent of 0.75 was derived based on morphologically found branching relationships [4].

The subendocardial layer of the heart contains a larger vascular volume compared to the midmyocardial and subepicardial layers [15]. This brings up the question of the influence of transmural differences on the scaling law. We have addressed this question by plotting the log-log relation between crown volumes with truncation radii of 50 and 200 respectively, similar as demonstrated in Fig. 4A. This was done for sub trees defined by the stem of penetrating transmural arteries where two groups were selected based on the depth the subtree reaches into the myocardial wall: Subendocardial trees reached to the true endocardium and the epicardial reached no further than 1/3 of the myocardial depth. For one heart the results are demonstrated in Fig. 7. There is a clear difference in relationship, indicating regional differences in the effect of truncation on crown volume. We could not establish reliable flow crown volume relations for these groups because the microsphere density was not sufficient to do so. However, Fig 7 indicates that regional differences on the flow-volume relationship are to be expected.

**Clinical application of scaling laws**

The method for measuring FFR where hyperemic flow is estimated from crown volume applying a scaling law has not arrived at the clinical stage yet. The feasibility of the method has been evaluated in animal experiments. Hyperemic flow was measured by first pass analysis of angiographic contrast [16] and the arterial tree was quantified from these angiographic images [1]. It should be noted that a value of 0.75 was assumed and used for data interpretation. Hence, the exponent was not determined from these experimental studies. Moreover, in these studies a region of interest for densitometry analysis is drawn in the images following the outlines of the larger epicardial arteries. Therefore, the crown volume measured by densitometry is only containing a selection of the coronary vasculature.
From our study the importance of selection of the crown is clear. The exponent for the scaling law is determined by the resolution of diameter measurement. It is of importance to note that in the experiments of Molloi et al. [17] where CT was used as imaging modality, the voxel resolutions amounted 200 μm. However, the smallest vessel diameter included in the analysis is much larger and in the order of 1 mm. Hence, in this case the truncation radius is more determined by vessels selection rather than image resolution.

Apart from the methodological aspects discussed, there are other serious limitations to be expected in the application of scaling laws. Hyperemic coronary flow is not only dependent on tree topology but also on cardiac function and coronary arterial pressure. Especially at the subendocardium the hemodynamic conduction is strongly dependent on diastolic time fraction and decreases with increasing HR and increasing with coronary pressure [18]. A detailed discussion of these aspects have been reviewed recently [19-21] and are outside the scope of this paper. Not only blood flow is dependent on conditions but blood volume as well. Arresting the heart at constant arterial pressure results immediately in an increase of intramural volume [22]. Moreover, in terms of evolution one may wonder why the design principle results in hyperemic flow and crown volume in the diastolic heart while normally the heart is beating and the flow limited to about 25% of the hyperemic value due to flow regulation.

It is more and more recognized the patients may suffer from microvascular disease [23]. It may be possible that microvascular disease becomes apparent from flow-crown volume relationships. With the recent improvements in imaging of perfusion and vascular structures by MRI and CT it is not impossible that flow-crown volume measurements may gain some clinical value in diagnosing microvascular disease.

**Study limitations**

Vascular casting may suffer from inconsequent filling of the vascular tree due to air bubbles and possible blood clots. Distributions of terminal segment radii were used to investigate the quality of overall filling. The datasets presented in this study were selected from a larger group based on their quality of uniform filling.

The choice of a circular cross-section may result into incorrect vessel volume measurements if the vessel cross section is in fact elliptical. The coefficient of variation was investigated for the each set of 64 radial measurements of the diameter per centerpoint and resulted into a value of 0.3 or lower for all segments. The low value of the coefficient of variation means that the vessels cross-section can safely be assumed circular.

A possible error in measured length of the segment can occur since the length of the vessel was defined as the Euclidian distance between start and endpoint of the segment. This can possibly underestimate the length in case of a bend vessel. For one dataset, summed centerpoint length and Euclidian length of the segment were plotted and showed a linear relation with a slope close to 1.
Conclusion

Cryomicrotome analysis allows simultaneous measurements of flow and vascular structure down to the microvascular level and therefore allows investigation into the scaling law behavior. The scaling law exponent $\beta$ depends on the truncation level of included vessel radii and a correct value is paramount for estimation of hyperemic blood flow. The scaling law exponent $\beta$ decreases when the truncation level of included vessel diameters is increased.

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