Assessment of the Degree of Coronary Stenosis Based on Forensic Pathological Autopsy

Yong Ke, Jun Ma, ZhenYuan Wang*

Abstract: Aims: The purpose of this project was to determine the percentage of the lumen area to the whole vessel area of normal coronary and stenotic coronary in humans at postmortem, to compare the difference between the value of measurement to coronary samples and slices, and finally to provide a reference for assessing the coronary stenosis severity.

Methods and Results: Image Analyze software was used to measure the circumference of 82 human normal coronary artery samples, and then the percentage of the lumen area to the whole vessel area was calculated. Total 134 human coronary artery samples and slices were imaged using camera and microscope. The lumen area sizes were measured using Motic Images Advanced 3.2 software, yield $R_{S1}$ and $R_{S2}$. The percentage of the lumen area to the whole vessel area of normal coronary artery is 52.1%±3.3%. There were obviously differences between $R_{S1}$ and $R_{S2}$.

Conclusions: The percentage of lumen area to the whole vessel area could be measured and calculated exactly using image analysis software, which can avoid the variability inherent in subjective estimates. The lumen area sizes of coronary slices measured with the image analyze software overestimated that of coronary samples by 7.9%±5.8%.

Key Words: sudden cardiac death, coronary stenosis, postmortem, forensic autopsy

According to incompletion statistics, about 6,000,000 cases of sudden death occur per year worldwide and 1,800,000 cases per year in China. Sudden death is unexpected death without any warning signs, which is an increasingly common mode of death around the world. Sudden death consists of predominantly sudden cardiac death (SCD), which accounts for 60% of all cases of sudden death[1]. SCD is estimated at approximately 50,000 cases per year in the United Kingdom and 300,000 ~ 400,000 cases per year in the United States[2]. Data from the Centers for Disease Control show that sudden cardiac death accounts for more than 460 000 deaths in 1999 in the United States, which is about two-thirds of all cardiac deaths in the United States[3]. In northern European populations the incidence of SCD is approximately 1 in1000 individuals[4]. Coronary artery disease (CAD) causes 70~80% of SCD[3,5]. Sudden death occurs suddenly and unexpectedly, and thus often leads to legal issues especially when there are precipitating factors involved such as emotion agitation, alcohol drinking, and the like.

One major task for the pathologist who performs autopsies is to ascertain the cause of death. The estimation of the degree of coronary stenosis is very important for determining if the death is caused by a preexisting cardiac lesion such as coronary stenosis, which can be assessed by lumen reduction or by the reduction in longitudinal diameter. The percentage stenosis is calculated by the ratio between the minimal lumen diameter resulting from the stenosis and the reference diameter upstream or downstream of the
lesion, identified as normal. However, it may be more accurate to estimate coronary stenosis by measuring the absolute lumen size of the vessel at autopsy [6].

This study was conducted to determine the percentage of the lumen area to the whole vessel area of normal coronary or stenotic coronary at postmortem using an image analysis software.

This work will be described in two parts for clarity. Part I was focused on studying the percentage of the lumen area to the whole vessel area of normal coronary, while part II was to measure the percentage of the lumen area to the whole vessel area of stenotic coronary. We believe that the severity of coronary stenosis could be assessed by comparing the data sets obtained from part I and part II, respectively. We also investigated the influence of tissue processing on the lumen area of coronary samples in part II.

**Part I**

**Methods**

We selected 82 human hearts with normal coronary artery supplied by the medicolegal centre of Xi’an Jiaotong University. All hearts were fixed in 10% formalin. The coronary samples (36 round shape and 46 oval shape) were taken from the left anterior descending coronary arteries (2 cm away from the left coronary orifice). The fixed samples were embedded in paraffin and tissue sections were cut and stained by hematoxylin and eosin. The image of the histologic slices were visualized with an imaging system (Moticam2306, 3.0M Pixel) connected to a microscope (Olympus, Primo Star). Then, the lumen area and circumference of the histologic slices were measured directly with Motic Imanges Advanced 3.2 software. The parameters of lumen and whole vessel were measured with bounds of intima and smooth muscle respectively (Figure 1).

Given that \( Cr_2 = 4\pi \times Sr \) (\( C \) represents circumference, \( S \) area, \( r \) round shape), it can be inferred that

\[
\begin{align*}
\frac{\text{lumen area}}{\text{whole vessel area}} &= \frac{\text{lumen circumference}}{\text{whole vessel circumference}}^2 \quad \text{(Formula 1)}
\end{align*}
\]

The ratio of the lumen area to the total vessel area can be calculated according to the Formula 1.

Using Motic Imanges Advanced 3.2 software, we obtained the lumen area and the total vessel area, respectively, and directly calculated the ratio of the lumen area to the total area, which yielded \( R_1 \). Similarly, we obtained the lumen circumference and the whole vessel circumference, and then calculated the ratio of the lumen area to the whole vessel area according to Formula 1, which yielded \( R_2 \).

Statistical analysis was performed using SPSS 13.0 software. The values of paired samples were assessed using the Student’s t-test. A \( P \) value <0.05 was considered statistically significant.

**Results**

In table 1, it can be seen that there is no difference between \( R_1 \) and \( R_2 \) when the lumen shape is round, but the difference is significant between \( R_1 \) and \( R_2 \) when the lumen shape is oval.

We measured the lumen circumference and the whole vessel circumference of 82 normal coronaries, and calculated the ratio of the lumen area to the total area. We analyzed the data and found that the mean ratio and the standard deviation were 0.521 and 0.033 respectively. Thus, the percentage of the lumen area...
ensure consistency, we selected the coronary samples from the same place (the left anterior descending coronary arteries, 2 cm away from the left coronary orifice).

As far as a single case was concerned, the samples were taken at autopsy not only from the left anterior descending coronary artery. We also took some normal coronary samples from the right coronary artery, left main coronary artery and left circumflex coronary artery and repeated the experiment. The data showed the same result, i.e., there was no significant statistical difference between this group result and the result mentioned in former paper (P <0.05).

Why normal coronary samples were only selected? We thought it’s more important to ensure the percentage of the lumen area to the whole vessel area of normal coronary as we estimated the severity of coronary stenosis. Most pathologists examine the cross-section of the coronary at necropsy, and then compare the size of the arterial lumen with a predicted normal lumen size for a vessel of that cross-sectional area. Thus, the predicted normal lumen size is apparently more important.

In normal situation, the blood flows unblocked in the vessel. When the shape of the vessel cross-section is round, blood flows with least resistance, because the round shape has the biggest area for a given circumference. The change of the vessel shape caused by any reason will result in an increase in the peripheral resistance. In vessel stenosis, the shape of the cross-section should approach maximal round to ensure the minimal flow resistance [7]. Pathological studies have previously shown that as plaque progresses, the plaques and the vessel bulge outward together [8]. This implies that in vivo the arterial lumen is round in shape. The slit-like and oval lumen shapes seen at autopsy are due to un-filled or collapsed artery [9]. The collapsed vessel results in decreased lumen area, but does not have any effect on the circumference. Thus, the vessel circumference could be used to assess the degree of coronary stenosis, as shown in Table 1.

At necropsy, most pathologists examine the cross-section of the coronary, either visually or by direct measurement, and then compare the size of the arterial lumen with a predicted normal lumen size for a vessel of that cross-sectional area. Although this method is often used in practice, it is subjective and not highly reliable for estimating the severity of coronary artery stenosis since the interobserver variability is high [10-15].

Mann JM [16] et al used a method similar to quantitative angiography to assess the severity of 31 human coronary arteries. Compared with this method, what is usually used by pathologists to measure coronary artery stenosis overestimates the severity of the lesion by 25-30%. The main reason for the overestimation is the remodeling of the vessel wall. Also, coronary artery is more circular in shape as it is in life, but the lumen collapses to an oval or slit shape at postmortem. So, it is of no practical value to compare the conventional pathology methods with the clinical methods in assessing the degree of coronary stenosis.

Methods
We studied 134 hearts from patients who died suddenly from heart disease. The coronary samples were imaged using camera (Olympus, μ1010), and then the slices were made.

The lumen circumference and the whole vessel circumference of coronary samples and slices were measured, and the ratios of area were calculated using Formula 1, which yielded $R_{s1}$ and $R_{s2}$.

Results
As shown in Table 2, $R_{s2}$ is larger than $R_{s1}$, indicating that the lumen area size increases due to the contraction of the vessel wall and plaque that occurs during tissue processing for H-E staining. In other words, the degree of coronary stenoses would be underestimated if the lumen size is measured on tissue sections. That is the main reason that the pathologists usually estimate the degree of coronary stenoses by Naked Eye in forensic practice.

<table>
<thead>
<tr>
<th>Lumen Shape</th>
<th>n</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round</td>
<td>36</td>
<td>0.018</td>
<td>0.013</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Oval</td>
<td>46</td>
<td>0.127</td>
<td>0.046</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$R_{s2}$-$R_{s1}$</th>
<th>n</th>
<th>Mean</th>
<th>Std.Deviation</th>
<th>P(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>134</td>
<td>0.079</td>
<td>0.058</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
The correlation between $R_{S1}$ and $R_{S2}$ was analyzed. There was good correlation between $R_{S1}$ and $R_{S2}$, and the correlation coefficient is 0.813.

Tissue slice is easy to observe and the lumen border is clearly defined when it is measured with imaging analyzing software. However, the value of visual assessment of the coronary lumen area size can be calculated using $R_{S2}$. We can see from Table 2, the mean of value is 0.079. There is good correlation between $R_{S1}$ and $R_{S2}$ ($r=0.813$). So, when the percentage of the lumen area of coronary slice to the total vessel area measured with imaging analyzing software is 43.3%, the percentage of the lumen area of coronary sample to the total vessel area is 35.4%.

**Discussion**

Sudden death, predominantly of cardiac origin, is an increasingly common mode of death around the world. Due to the unexpected nature, sudden death often triggers legal issues especially when there are precipitating factors involved such as emotion agitation, alcohol drinking, and the like. Therefore, it is extremely important to precisely determine the degree of coronary stenosis and to evaluate if and to what extent the identified coronary stenosis contributes to the death.

In the present study, we described a new and more accurate approach to assess the severity of coronary stenosis, which should have important implications in legal medicine practice. Specifically, the sizes of lumen area of 134 coronary samples and tissue slices were measured, and the difference between them was analyzed. Our results indicate that the lumen area size increased due to the contraction of the vessel wall and plaque in the process of making histologic slice, as shown by $R_{S2}>R_{S1}$. There was good correlation between $R_{S1}$ and $R_{S2}$ ($r = 0.813$) and the mean value was 7.9%. Thus, the percentage of the lumen area of coronary sample to the total vessel area could be inferred by the percentage of the lumen area of coronary slice to the total vessel area.

It is clear that the visual estimation of stenosis severity by a single observer does not necessarily produce reliable results. Although the mean value of multiple visual estimates is more reliable than a single value from a single observer, the visual estimation is still not as accurate as the quantitative method. Also obtaining multiple observations is not practical in real world [17]. Moreover, many researches have confirmed that the error of the degree of stenosis measured with instrument was less than that of visual assessment even if it is estimated by multiple observers [17-20].

J. Staiger et al [21] measured 203 coronary artery cross-sections using quantitative planimetric method, and compared the values with the results of postmortem coronary angiograms. A highly significant difference between the angiographic and morphologic findings was detected. In the study of Mann JM et al [16], 31 human hearts were perfused with 10% formal saline. Stenosis indexes were calculated by comparing the lumen diameter of the stenotic region with the lumen diameters in adjacent normal arterial segments. The coronary artery segments were processed histologically, and stenosis was remeasured by comparing the lumen diameter with the diameter of the vessel within the internal elastic lamina. The authors found that the method used on histological slides overestimated the degree of stenosis by 25-30%. During the development of atherosclerotic plaque, to maintain the lumen area size, the media of the coronary artery undergoes focal atrophy and the vessel bulges outwards, which results in increases in the vessel external diameter [16,22,23]. Many researchers have applied various methods to study the lumen area size and degree of stenosis to mimic physiological condition without satisfaction, because the function of the vascular smooth muscle and internal elastic lamina is lost at postmortem, which can impact vessel remodeling [24-26].

This study was focused on an objective and accurate method that can be used to assess the severity of coronary stenosis at and after autopsy. The information regarding the degree of the coronary stenosis can be very useful when it comes to determining the cause of sudden death. However, there are many more factors that need to be considered to tease out the true cause of death, especially when other pathological changes are observed at autopsy. Thus, diameter of the vessels alone does not necessarily prove causation. If there is interstitial fibrosis or old healed infarct, we have to weigh out the relative importance of these factors in causing death as compared with coronary stenosis. The degree of the coronary stenosis is normally divided into four grades based on the percentage of the atheromatous plaque area to the lumen area. A percentage of 0~25% is classified as grade I, 26~50% grade II, 51~75% grade III, and 76~100% grade IV. The grade
IV could be considered causative in practice. However, when coronary stenosis is under grade III, we need to rule out other possibilities before we conclude that the death is caused by coronary stenosis. It should be noted that at present, the percentage of coronary stenosis mentioned here is visually estimated by the pathologist. In summary, the determination of the cause of death is a systemic and complex process. Besides coronary stenosis, many other factors should also be considered. Sometimes, death could be caused by multiple factors.

**Conclusions**

*In vivo*, the arterial lumen is round in shape as the vessel is filled with blood. The artery lumen is emptied and collapsed at postmortem, making it slit-like or oval shaped. It is difficult for pathologists to visually convert the area of an oval or slit-shaped lumen to what it would be in life. The problem could be resolved if the lumen circumference was used to assess the severity of coronary stenosis.

The coronary lumen circumference and the whole vessel circumference could be measured accurately with imaging analyzing software, and then, the percentage of the lumen area to total vessel area could be calculated. This could avoid the inherent error from visual estimation.

In the present study, we found the mean percentage of the lumen area to the whole vessel area of normal coronary slice was 52.1%, and the lumen area increased about 7.9% after tissue processing. So, we can infer the severity of coronary stenosis according to Formula 2.

\[
n = \left(1 - \frac{X - 7.9\%}{52.1\% - 7.9\%}\right) \times 100\%
\]

(Formula 2)

For example, if the percentage of the coronary lumen area to the total area measured and calculated with the image analysis software were 28.9%, the degree of coronary stenosis would be 51.5%.

It has to be pointed out that although we believe the method described will be applicable in most cases, it may not be feasible when the vessel intima or the vessel wall is fragmented, which makes it difficult to accurately measure the lumen circumference and the whole vessel circumference.

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**References**