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# Comparison of very-high-frequency ultrasound assessment of radial arterial wall layers after first and repeated transradial coronary procedures

Bei-Bei ZHANG, Yu-Jie ZHOU, Jie DU, Shi-Wei YANG, Zhi-Jiang WANG, Hua SHEN, Zhi-Ming ZHOU

*Bejing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart Lung and Blood Vessel Disease, Beijing, China*

**Abstract**

**Background** Transradial coronary procedure (TRP) traumatizes the radial artery (RA), especially resulting in changes to arterial wall morphology. This study explored the significance of the early onset of traumatic effects to wall layers of the RA following the first TRP (FTRP) and repeat TRP (RTRP) using very-high-frequency ultrabiomicroscopy (VHFUBM). **Methods** A total of 1431 patients that received TRP were divided into the FTRP group that comprised 781 patients and the RTRP group that comprised 650 patients depending on the number of procedures. Two-dimensional RA images were acquired by 30–55 MHz ultrasound one day before and one day after the procedure. **Results** After TRP, the incidence of intimal tears, medial dissections and external elastic lamina fracture were greater in the RTRP ($P < 0.001$). The RTRP group showed significantly thicker intimal thickness (IT), media thickness (MT), adventitia thickness and all complex layer thicknesses as compared with the FTRP group ($P < 0.001$). **Conclusions** Multivariate linear regression analysis discovered that repeated TRP and other observations were independent predictors of increased IT in post-operative RA. VHFUBM provides an approach to study structural and histopathological injury in the wall layers of RA which showed increased trauma to the RA following RTRP.

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**Keywords:** Radial arterial wall morphology; Transradial procedure; Very-high-frequency ultrabiomicroscopy

## 1 Introduction

Twenty years has elapsed since the first transradial coronary procedure (TRP) was successfully performed by Kiemeneij and Laarman.[1] Since then, this method has been increasingly adopted by the international community and especially in Asia and some European countries, due to its superior features of lower vascular complications, shorter hospitalization times, and decreased costs, decreased discomfort and improved quality of life for patients as compared with transfemoral procedures (TFP).[2–6] However, trauma to the radial artery (RA) induced by TRP might influence its function, lead to stenosis and occlusion, increased the difficulty associated with repeated TRP, decreased utilization of the RA as a conduit for coronary arterial by-bass grafting and limited the success and future utility of the TR approach.[7–10] Thus, the study of morphological changes to the RA wall layers post-surgical intervention is very important. In addition, although various imaging techniques such as intravascular ultrasound, optical coherence tomography and computed tomography angiography were applied to RA, they were otherwise invasive approaches or associated with radiation.[11–13] Repeated TRP would cause multiple injuries to the RA during surgical procedures associated with increased failure rates that were mainly due to chronic and progressive stenosis and block of the radial artery.[14] Many studies demonstrated early injuries to the RA in patients that had undergone repeat TRP, and these injuries exceeded those that were seen following the first TRP on comparing lumen diameter, lumen or various layers of the membrane area and measures of the intimal-medial thickness (1MT).[15,16] Thus, the study of morphological changes to the RA wall layers post-surgical intervention is very important. In addition, although various imaging techniques such as intravascular ultrasound, optical coherence tomography and computed tomography angiography were applied to RA, they were otherwise invasive approaches or associated with radiation.

Using standard-frequency (7–15) MHz ultrasound with a resolution to 200–400 μm,[10] the RA wall structure is difficult to evaluate.[17] Conventional ultrasound can only observe the lumen of the RA and measure intimal-media thickness only for clear intimal thickening, hence each layer of the membrane could not be observed and measured in detail. In the current study, we present a non-invasive very-

**Correspondence to:** Yu-Jie ZHOU, MD, Bejing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart Lung and Blood Vessel Disease, Beijing 100029, China. E-mail: azzyj12@163.com
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high-frequency ultrasound imaging method to assess intima, media, adventitia and total wall thickness of RA that is beyond the resolution of conventional ultrasound. With ultrabiomicroscopy (UBM), all layers of the RA can be accurately measured.

Very-high-frequency ultrabiomicroscopy (VHRUBM) was originally developed for experimental studies of small animals with transducers in the 30–55 MHz range that permitted imaging of more superficial tissues at near microscopic detail. Moreover, a 40-MHz transducer provides a theoretic maximal axial resolution of approximately 30 μm and a lateral resolution of 62 μm.[18] In addition, a 55-MHz transducer provides a theoretic maximal axial resolution of approximately 20 μm.[19] In human subjects, preclinical research studies discovered that it was possible to obtain superior quality images and precise quantification of human superficial arteries, veins or nerves to a sub-dermal depth down to 20 mm.[20–23]

We hypothesized that the intima-media-adventitia thickness (IMAT) complex can be separated into measurements of intima, media and adventitia thickness (IT, MT and AT). Furthermore, the internal elastic lamina (IEL) and the external elastic lamina (EEL) can be observed clearly in individuals after TRP by VHRUBM. Few previous studies have performed detailed in vivo measurements of every layer in the RA wall, and no study has conducted studies of individuals that have previously undergone TRP.

The aim of this study was to explore previously undescribed insights into the potential for and mechanisms of early injury of the RA following TRP by studying changes in all wall layers.

## 2 Methods

This study was approved by the Institutional Research Ethics Board of Beijing-Anzhen Hospital, Capital Medical University. The VHRUBM equipment was used following the assistance of the Vascular Biology Department at the Beijing Institute of Heart Lung and Blood Vessel Disease. Written informed consent was obtained from each participant before entry into the study. A total of 1430 consecutive patients who had undergone TRP including transradial angiography and transradial intervention at the 12$^{th}$ Department of Beijing-Anzhen Hospital were retrospectively studied from February 2015 to March 2016. We excluded patients that had a negative Allen tests, peripheral vessel disease, radial arteries with anatomic abnormality and small lumen (average diameter of less than 2.0 mm), previous hemodialysis and surgical trauma of the forearm arteries. We also excluded patients that had previously presented with radial arterial thrombus. All study patients were divided into two groups depending on the procedural times; i.e., the first TRP group (FTRP) and the repeat TRP group (RTRP).

### 2.1 Clinical and procedural data

Clinical and procedural characteristics data included age, body mass index, HbA1c, percentage of the subjects, current smoking habits, and histories of diabetes, hypertension, and dyslipidemia, multiple puncture, transradial angiography (TRA) or transradial intervention (TRI), and multivessel disease, medication was also included. Current smoking was defined as smoking cigarettes every day or on some days of a typical week.[24]

### 2.2 Ultrasound measurements

#### 2.2.1 Radial arterial images

The RA was examined by very-high-frequency ultrabiomicroscopy using a 30–55 MHz transducer (Vevo2100 Visualsonics, Toronto, Canada). The highest frequency probe was applied to clearly view images of the far wall. If the far wall was not observed because of attenuation of the ultrasound beam in deeper tissues, the transducer was stepwise changed to one with a lower frequency. If the far wall of the vessel was not observed with a 30 MHz tranducer, the near wall was studied. Images of three types of frequency transducer are shown (Figure 1).

Every participant was examined one day before and one day after the procedure. Patients were examined in the sitting posture with right forearm abduction and at a level as high as the chest with the hands slightly relaxed. In addition, long-axis and short-axis views of the RA were acquired with the probe perpendicular to the artery. At the position of the thickest part of the far wall, digital cine-loops in B-mode of four consecutive beats were recorded in real time and stored. Analyses were then made off-line by the same investigator using electronic calipers and the ultrasound's distance measurement tool. The image analysis software of Vevo 2100 was validated (personal communication with Visual Sonics Inc., Canada), which allowed accurate measurement of vessel thickness, diameter and area. Measurements were performed in systole when the artery showed its largest diameter. These measurements were repeated on three different images, which were then averaged to give the values of each blood vessel in each subject.

#### 2.2.2 Ultra-measured parameters and caliper definitions

Calculated measurements in punctured blood vessels included the incidence rate of the plaque, intima media thickness, luminal stenosis ≥ 30%, thrombosis, occlusion, small

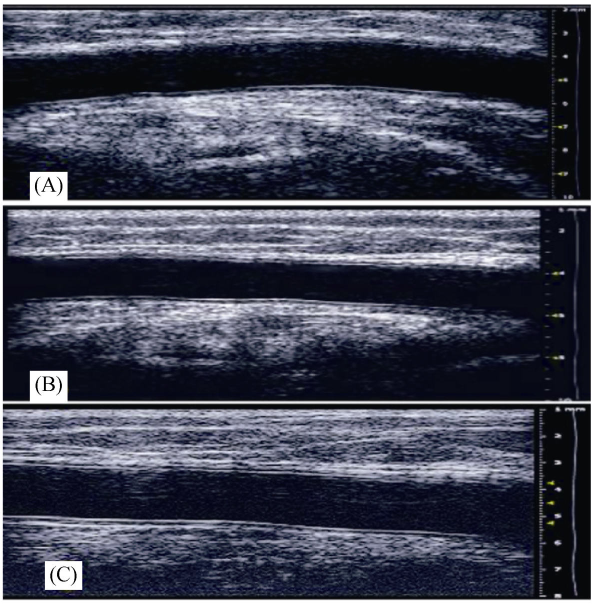


**Figure 1. Comparison of three types of high-frequency images of radial arteries in three subjects.** A representative image at 30 MHz (A); at 40 MHz (B); and a representative image at 55 MHz (C). The images illustrate significant differences in image quality, and the wall layers are best seen at the highest frequency.

RA (inner diameter < 2.0 mm), the ratio of the external sheath diameter and vessel diameter > 1, intimal or medial tear, EEL fracture and pseudo-aneurysm. Occlusion was accompanied by disappearance of the forward blood flow or reversal of the distal blood flow.

Luminal parameters included minimum luminal lumen diameter (MLD); minimal luminal area (MLA). Lumen diameter was defined by the distance between the intima–lumen interface of the near wall and the lumen–intima interface of the far wall.[25] The lumen area was defined as the minimum luminal area around the intraluminal boundary. The vessel cross- sectional area was measured in the short-axis view using a closed-loop freehand tracing tool of the vascular boundary and the software calculating area. The external elastic lamina area (EELA) was not routinely measured since the measurements were greatly affected by image quality, especially to the extent of diminished image clarity following surgery.

Layer observed items (from the inside to the outside) include intimal thickness (IT); internal elastic lamina (IEL); medial thickness (MT); intimal-medial thickness (IMT); the external elastic lamina (EEL); the adventitia thickness (AT); the IMAT; the external vessel wall border (EVWB). IT was defined as the distance from the lumen-intima interface to the interface of the intima-media of the far wall; IMT was defined as the distance from the luminal-intimal interface of the far wall to the edge of the medial-adventitial interface.[14] MT was defined as the distance from the leading edge of the IEL to EEL, the IEL and EEL together with the elastic fibers were in the media; AT was the distance from the edge of the EEL to the interface between the external wall border and the surrounding tissue. The increased rate of the IT was the ratio of the D-value of the post-operative IT minus the pre-operative IT divided by the pre-operative IT. Figure 2 displays longitudinal and cross-sectional ultrasound examination of the RA by VHFUBM.

Every radial arterial parameter was measured three times on the same occasion by the same single deputy chief ultrasonic physician, following which the Concordance Correlation Coefficient (CCC) on the intra-observer agreement was summarized.

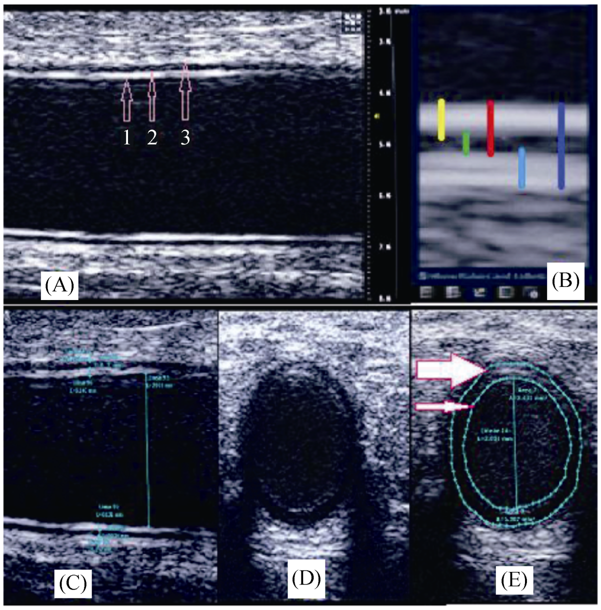


**Figure 2. Longitudinal and cross-sectional examination of RA.** (A): RA wall with a triple line pattern composition of the following: (1) IEL; (2): EEL; and (3) EVWB. (B): A measuring tool. Morphological measurements include IT (yellow mark), MT (green mark), IMT (red mark), AT (light blue mark) and IMAT (blue mark). (C): Measurements of lumen diameter and various layers of the membrane thickness in long axis section. (D): RA image in short axis section. (E): the measurements of the minimum luminal area MLA (as indicated by the thin arrow), and the EELA (as indicated by the thick arrow). AT: adventitia thickness; EEL: external elastic lamina; EELA: external elastic lamina area; EVWB: external vessel wall border; FTRP: the first transradial coronary procedure; IEL: internal elastic lamina; IMAT: intima-media-adventitia thickness; IMT: intima-media thickness; IT: intima thickness; MLA: minimum luminal lumen area; MT: media thickness; RA: radial artery.

### 2.3 Transradial coronary procedures

In this procedure, 1 mL of 1% lidocaine was subcutaneously injected for local anesthesia, then the right or left RA was punctured using a 20-gauge needle and a 0.635 mm (0.025 inch) hydrophilic guidewire (TERUMO Co., Tokyo, Japan) was inserted through the needle. The sheath size used was 6F–7F (external diameter 2.48–2.68 mm). With the sheath in place, 0.2 mg of nitroglycerin and heparin (5000 IU for percutaneous coronary angiography or 10000 IU for percutaneous coronary intervention) were introduced. After completing the transradial coronary procedure, the arterial sheath was removed and hemostasis was achieved by RA hemostasis device placement over the artery, each of which was applied as a one-time 4 h decompression, followed by bandage compression of the punctured site.

### 2.4 Statistics

Statistical analyses were made by The Statistical Package for Social Science, SPSS version 13.0 (Chicago, IL, USA). Different groups were compared by the Student's *t*-test for continuous variables and the Chi-square or Fisher's exact test for categorical variables. The Wilcoxon signed rank-sum test was used for variables that were non-normally distributed. Multivariate linear regression analysis was also performed. Evaluation of intra-observer consistency was analyzed by MedCalc software. An alpha value of $P < 0.05$ was considered statistically significant.

## 3 Results

A total of 1431 consecutive patients (age 58.7 ± 9.4 years, 423 females and 1008 males) underwent TRP. Among them, 763 received TRA and 668 received TRI. All study patients were divided into two groups: 781 patients comprised the first TRP (FTRP) group, and 650 patients comprised the repeat TRP (RTRP) group.

### 3.1 Clinical and procedural characteristics

The clinical and procedural characteristics of patients that received FTRP and RTRP are shown in Table 1. There were more cases that presented with a history of diabetes (55.7% *vs.* 34.8%, $P < 0.001$) and multi-vessel disease (65.1% *vs.* 33.3%, $P < 0.001$) in the RTRP group than were found in the FTRP group; whereas cases that had current smoking habits (42.5% *vs.* 36.8%, $P < 0.05$) and received transradial angiography (66.7% *vs.* 37.2%, $P < 0.001$) in the FTRP group were highest in the RTRP group. The groups did not differ significantly with respect to age, body mass index, and HbA1c in the study ($P > 0.05$). There was also no significant difference between either group by gender, histories of hypertension, dyslipidemia, and multiple punctures ($P > 0.05$). Medications such as renin-angiotensin system inhibitors [angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB)], statins, and antiplatelet drugs were taken with more cases in the RTRP group than in the FTRP group.

**Table 1. The basic characteristics of the participants.**

| Variable | FTRP ($n$ = 781) | RTRP ($n$ = 650) | Statistics ($t$ or $X^2$) | $P$-value |
|---|---|---|---|---|
| Age, yrs | 58.5 ± 9.3 | 59.0 ± 9.5 | $t = -0.227$ | 0.821 |
| Sex | | | $X^2 = 0.493$ | 0.482 |
| Male | 555 (71.1%) | 453 (69.7%) | | |
| Female | 226 (28.9%) | 197 (30.3%) | | |
| Body mass index, kg/m$^2$ | 25.9 ± 2.7 | 26.4 ± 3.4 | $t = -0.679$ | 0.509 |
| Current smoking | 332 (42.5%) | 239 (36.8%) | $X^2 = 6.072$ | 0.014* |
| Diabetes history | 272 (34.8%) | 362 (55.7%) | $X^2 = 81.767$ | < 0.001** |
| Hypertension history | 394 (50.5%) | 353 (54.3%) | $X^2 = 2.873$ | 0.090 |
| Dyslipidemia history | 326 (41.7%) | 300 (46.2%) | $X^2 = 3.603$ | 0.058 |
| HbA1c | 6.4% ± 1.0% | 6.9% ± 2.2% | $t = -1.133$ | 0.262 |
| TRP | | | $X^2 = 167.474$ | < 0.001** |
| TRA | 521 (66.7%) | 242 (37.2%) | | |
| TRI | 260 (33.3%) | 408 (62.8%) | | |
| Multi-vessel disease | 260 (33.3%) | 423 (65.1%) | $X^2 = 194.519$ | < 0.001** |

Data were presented as mean ± SD or $n$ (%). *$P < 0.05$, **$P < 0.001$. FTRP: first transradial coronary procedure; HbA1c: glycoseylated hemoglobin A1c; RTRP: repeat transradial coronary procedure; TRA: transradial angiography; TRP: transradial coronary procedure; TRI: transradial intervention.

### 3.2 Ultrasound results by VHFUBM

No adverse effects of VHRUBM were observed. Pre-operative examination and post- operative complications are summarized in Table 2. Before the procedure, the incidence of intima media thickening in the RTRP group was greater than that found in the FTRP group (19.4% *vs.* 9.5%, $P < 0.001$), neither group differed in respect of the percent of plaques, RA inner diameter < 2.0 mm, external sheath diameter/vessel diameter > 1 ($P > 0.05$).

After TRP, intima media thickening was extremely common, difference between either group was significant (89.4% *vs.* 80.2%, $P < 0.001$). The incidence of luminal stenosis ≥ 30% (23.8% *vs.* 9.3%, $P < 0.001$) and luminal occlusion (3.1% *vs.* 1.3%, $P < 0.001$) in the RTRP group was greater than that found in the FTRP group, whereas the incidence of thrombosis was not significantly different between groups ($P > 0.05$). Obliteration usually occurred within approximately a 5–10 cm distance from the puncture site.

**Table 2. Preoperative examination results and postoperative complications around puncture point observed by VHFUBM.**

| Variable | FTRP (*n* = 781) | RTRP (*n* = 650) | Statistics (*t* or $X^2$) | *P*-value |
|---|---|---|---|---|
| Preoperative examination | | | | |
| Plaque | 24 (3.1%) | 32 (4.9%) | $X^2 = 3.297$ | 0.069 |
| Intima media thickness | 74 (9.5%) | 126 (19.4%) | $X^2 = 31.327$ | < 0.001** |
| Lumen stenosis ≥ 30% | 0 | 55 (8.5%) | | - |
| RA inner diameter < 2.0 mm | 28 (3.6%) | 36 (5.5%) | $X^2 = 3.244$ | 0.072 |
| External sheath diameter/vessel diameter > 1 | 161 (20.6%) | 159 (24.5%) | $X^2 = 3.412$ | 0.065 |
| Postoperative complications | | | | |
| Intima media thickening | 626 (80.2%) | 581 (89.4%) | $X^2 = 39.948$ | < 0.001** |
| Lumen stenosis ≥ 30% | 73 (9.3%) | 155 (23.8%) | $X^2 = 61.032$ | < 0.001** |
| Thrombosis | 10 (1.3%) | 56 (8.6%) | $X^2 = 4.434$ | 0.543 |
| Occlusion | 10 (1.3%) | 20 (3.1%) | $X^2 = 5.642$ | 0.018* |
| Intimal tear | 230 (29.4%) | 314 (48.3%) | $X^2 = 67.046$ | < 0.001** |
| Media dissection | 92 (11.8%) | 196 (30.2%) | $X^2 = 83.840$ | < 0.001** |
| EEL fracture | 83 (10.6%) | 151 (23.2%) | $X^2 = 45.202$ | < 0.001** |

Data were presented as mean ± SD or *n* (%). **P* < 0.05, ***P* < 0.001. EEL: external elastic lamina; FTRP: the first transradial coronary procedure; RA: radial artery; RTRP: repeat transradial coronary procedure; VHFUBM: very-high-frequency ultra biomicroscopy.

The incidence of intimal tears, medial dissection and EEL fracture were greater in the RTRP group than in the FTRP group (intimal tear: 48.3% *vs.* 29.4%, *P* < 0.001; medial dissection: 30.2% *vs.* 11.8%, *P* < 0.001; and EEL fracture: 23.2% *vs.* 11.6%, *P* < 0.001). Figure 3 displays abnormal ultrasound image of the radial artery by VHFUBM.

Adequate images of the radial arterial far wall were obtained by VHFUBM in our study. Consistent evaluation that was determined for intra-observer effects was excellent for all measurements, with an CCC of 0.97 (0.94 and 0.99) for the IT; 0.95 (0.91 and 0.99) for the MT; 0.93 (0.85 and 0.97) for the AT; 0.98 (0.95 and 0.99) for the MLD; and 0.92 (0.84 and 0.97) for the MLA, respectively.

The study groups did not differ with regard the depth of skin close to the wall. Individuals included in the RTRP group showed decreased MLD and MLA as compared with the FTRP group (*P* < 0.001). Individuals in the RTRP group had significantly increased IT, MT, AT, IMT and IMAT as compared with the FTRP group (*P* < 0.001); furthermore, the intima/media index is different between either group (*P* < 0.001, Table 3).

An increased rate of IT was considered a dependent variable. Multivariate linear regression analysis discovered that repeated TRP, external sheath diameter/vessel diameter < 1 and multi-vessel disease were clinically independent predictors of increased rates in IT. After controlling for clinical and procedural variables, intimal tear and medial dissection were independent clinical predictors of increased IT rate in post-operative RA. No significant association was found between medications such as ACEI, ARB, statins, and anti-platelet drugs and any increased rate of IT (Table 4).

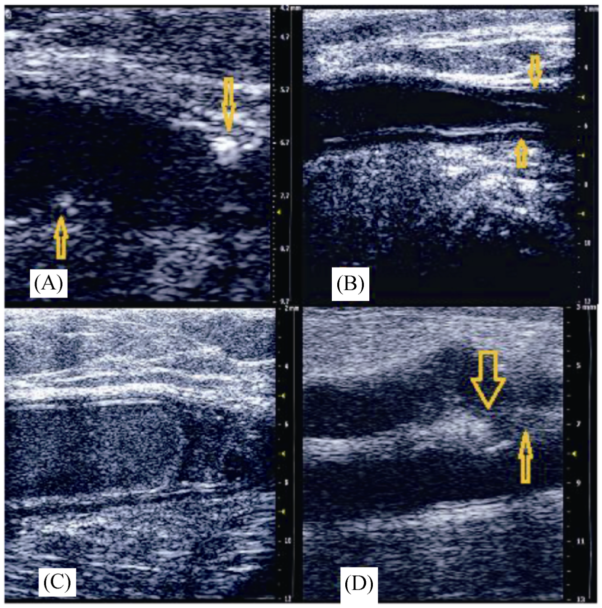


**Figure 3. Displays abnormal images of the RA.** (A): an experimental image of plaques (as indicated by arrows) on the vascular wall of RA; (B): an image of a luminal stenosis with significantly increased IMT (as shown by the arrow); (C): an image of typical obliteration by thrombosis in RA; and (D): an image of intimal media tear (as indicated by a thin arrow), and EEL shows echo disappearance (i.e., as shown by the thick arrow). EEL: external elastic lamina; IMT: intima-media thickness; RA: radial artery.

## 4 Discussion

Radial artery catheterization for coronary interventions is

**Table 3. Ultra measurements around puncture point of postoperative RA in FTRP group and RTRP group using VHFUBM.**

| Variable | FTRP ($n$ = 781) | RTRP ($n$ = 650) | Statistics ($t$ or $X^2$) | $P$-value |
|---|---|---|---|---|
| MLD, mm | 1.54 ± 0.79 | 1.31 ± 0.54 | $t$ = 6.531 | < 0.001** |
| MLA, mm$^2$ | 2.54 ± 0.78 | 1.68 ± 0.69 | $t$ = 13.087 | < 0.001** |
| Depth skin to near wall, mm | 5.70 ± 1.50 | 5.57 ± 1.25 | $t$ = 1.792 | 0.073 |
| IT thickness, mm | 0.23 ± 0.13 | 0.40 ± 0.13 | $t$ = –20.675 | < 0.001** |
| MT thickness, mm | 0.13 ± 0.05 | 0.14 ± 0.05 | $t$ = –4.510 | < 0.001** |
| IMT thickness, mm | 0.41 ± 0.16 | 0.53 ± 0.13 | $t$ = –15.423 | < 0.001** |
| I/M index | 1.92 ± 0.80 | 3.41 ± 1.37 | $t$ = –16.070 | < 0.001** |
| AT thickness, mm | 0.19 ± 0.05 | 0.22 ± 0.09 | $t$ = –8.255 | < 0.001** |
| IMAT thickness, mm | 0.55 ± 0.14 | 0.71 ± 0.19 | $t$ = –17.549 | < 0.001** |

Data were presented as mean ± SD. *$P$ < 0.05, **$P$ < 0.001 AT: adventitia thickness; FTRP: first transradial coronary procedure; I/M index: intima/media index; IMAT: intima-media-adventitia thickness; IMT: intima-media thickness; IT: intima thickness; MLA: minimum luminal lumen area; MLD: minimum luminal diameter; MT: media thickness; RTRP: the repeat transradial coronary procedure; VHFUBM: very-high-frequency ultrabiomicroscopy.

**Table 4. Predictors of the IT increased rate around the puncture point by multivariate linear regression analysis.**

| Variable | B | SE | $t$ | $P$-value |
|---|---|---|---|---|
| Repeat-TRP | 2.253 | 0.693 | 3.252 | 0.003* |
| Female gender | 1.656 | 0.607 | 0.482 | 0.012* |
| TRI | 2.690 | 1.327 | 2.028 | 0.054 |
| Sheath diameter/vessel diameter > 1 | 1.605 | 0.612 | 2.624 | 0.015* |
| Multi-vessel disease | 2.660 | 0.993 | 2.680 | 0.013* |
| Intimal tear | 6.114 | 1.378 | 4.438 | 0.001* |
| Media dissection | 1.656 | 0.607 | 2.728 | 0.012* |

IT indicates that the increased rate is defined as the ratio of the D-value of the post-operative IT minus the pre-operative IT divided by the preoperative IT. *$P$ < 0.05, **$P$ < 0.01, regression analysis was statistically significant. IT: intima thickness; TRI: transradial intervention; TRP: the transradial coronary procedure.

potentially associated with short and long-term effects, which break the integrity of the RA luminal wall.[26] Yan, *et al.*,[27] measured the RA intima-media thickness, one day and one month after TRP, results showed the mean radial artery IMT increased from 0.25 ± 0.12 mm before the procedure to 0.69 ± 0.31 mm one day post procedure ($P$ < 0 .01) and decreased to 0.38 ± 0.17 mm 1 month following the procedure ($P$ < 0 .05 compared with baseline).

Our research is the first study that has reported using VHFUBM to evaluate the acute effects of TRP on each layer of the membrane of the RA. In the current study, we explored the mechanisms of the potential for early injury of the vessel wall. The findings from this report were as follows: (1) the incidence of intimal tears, medial dissection and EEL fracture in the RTRP group was greatest; (2) all layers of membrane of IT, MT, AT, IMT and IMAT showed more thickening in the RTRP group; (3) female, repeat-TRP, external sheath diameter/vessel diameter > 1, multi-vessel disease, intimal tear and media dissection were independent predictors of the growth of IT after TRP. The results of our study conveyed information that multiple puncture as well as insertion of the sheath during RA intervention were the main causes of injury. The danger becomes greater with repetitive catheterizations where the injury may not recover.[26]

Moreover, VHFUBM assists our exploration of the early potential trauma mechanism of RA that had undergone TRP by providing information on the pathogenic changes of the arterial wall layers. These results could be useful as a tool to more thoroughly detect radial artery structure prior to CABG, PCI or fistula operation.

Previous studies have validated the accuracy and precision of both *ex vivo* and *in vivo* approaches by VHFUBM. Studies of the UBM against silicone layers of different thickness and histological assessment of human superior mesenteric artery informed that UBM supplied accurate measurements *ex vivo*.[28] Another experiment showed a significant correlation between histology and *in vivo* measurements of UBM of the rat carotid arterial IT.[29] Assessment of IMT, AT and IMAT in muscular arteries as determined by VHFUBM was precise. By contrast, traditional high frequency ultrasound was accurate but only for IMT of the large muscular and elastic arteries.[20] Contrary to some previously published studies,[28,30] an assessment of IT was over-estimated by VHRUBM.[20]

Following RTRP, the incidence of intima media thickening, luminal stenosis ≥ 30% and occlusion was greater than observed for the FTRP group. Individuals in the RTRP group showed decreased MLD and MLA as compared with the FTRP group. These observations are consistent with previous studies demonstrating greater trauma to the RA, which more easily and subsequently led to a decrease in luminal diameter with decreased area, stenosis, and even occlusion that resulted from repeated TRP.[31,32]

In the present study, we found that each layer of membrane IT, MT and AT of the RA were clearly elevated following RTRP. Moreover, total layers of membrane IMT and IMAT of RA were elevated more obviously after RTRP. The incidence of both intimal tear and medial dissection were highest in the RTRP group as compared with the FTRP group. Multiple puncture as well as insertion of the sheath affected the structure of the vessel wall.

The adventitia has been considered a primary element for detection of arterial injury. It can reflect different external and endogenous stimuli by initiating and regulating changes in the vascular endothelium.[33] Shi, *et al*.,[34] described this affect following injury by procedure, in which the adventitia of the porcine coronary artery is thickened and rich in muscle fibroblasts and collagen fibers (a phenomenon that generally occurs in the first three days of PCI).

Finally adventitia fibroblast cells (AF) underwent rapid apoptosis before arterial contraction, which resulted in scar formation.[33] During stages of vascular injury, adventitia fibroblast cells migrate from the adventitia through the media to the intima, which stimulates vascular remodeling of the media and intima.[34] Ultimately vascular constriction remodeling would become apparent. Negative remodeling caused by adventitial remodeling might represent a key biological determinant of vascular stenosis, which may be due to intimal hyperplasia, elastic recoil and microvascular regeneration.[35] By contrast, intimal hyperplasia is one of the main pathological mechanisms of vascular stenosis. When vascular endothelial cells are damaged, the vessel's natural protective barrier is subsequently disrupted. Moreover, the proliferation of vascular smooth muscle cells (SMC) is promoted and their migration from the media to the intima, which generally occurs within 1–3 days after vascular injury, finally results in neointimal formation.[36]

In the current study, we observed that repeat-TRP, external sheath diameter/vessel diameter < 1, and multi-vessel disease were independent clinical predictors of an increased rate of IT. Results were similar to previously published studies.[33,34,37,38] After controlling for clinical and procedural variables, intimal tear and media dissection were independent predictors of the increased rate of IT. When the IEL defective ratio was greater than 20% or the length greater than 3–4 μm, the intimal thickness increased.[39] Stable internal elastic lamina that behaves as an authentic barrier to the migration of SMC to the intima, can enhance the response to elevated tensile stress in the arterial wall.[40,41] The complete medium film and external elastic lamina represent effective barriers to prevent wide scale activation and migration of AF, which play important roles in the repair of moderately injured vascular tissue (i.e., media rupture). In the range of media integrity, the adventitia and intimal hyperplasia is not obvious.[42] Studies showed that use of statins and renin angiotensin system blockers for prolonged periods might affect the structural integrity of the vascular wall in patients with coronary heart disease.[43,44] However, associations between medication use and an increased rate of IT in post-operative RA was not found to be significant in our current research. A likely reason for this might be dependent on a subtle or less than obvious induction of acute injury of the vascular wall by select drugs.

From observations derived from our present study, we found a greater thickness for both MT and AT in the RTRP group, which was similar to that for IT. Furthermore, we derived a hypothesis that multiple punctures and insertions of the sheath not only brought about acute injuries to the intimal membrane, but also induced comparative injury to the medial and adventitia membranes. Transradial cardiac catheterization results in structural and histopathological injury to the wall of the radial artery, and the relative size of the introduced sheath in comparison to the inner luminal diameter. In addition, repeated TRP is associated with intimal hyperplasia. Efforts using smaller diameter guide catheters, improving procedural techniques, and employing pharmacological interventions may minimize radial wall injury.[16,45]

This study has some limitations. First, although VHRUBM accuracy is good for vascular layer thickness overall, it would be challenged since the thickness is closed to the limit of ultrasound resolution (30 μm). Also, the observed results of intimal tears, medial dissection and EEL fractures would be affected by acoustic beam interference, and those lesions could be hardly discovered under conditions where the range of lesions did not exceed 30 μm. Luminal area as measured in short-axis view would be influenced by lateral echo loss. In addition, due to penetration depth being limited to a fixed focal zone of 4.5 mm below the surface of the skin (55-MHz transducer), VHRUBM cannot produce high-quality images for individuals with post-surgical subcutaneous bruising. The current study was conducted to investigate changes of all the wall layers of RA one day after being injured by the first-TRP and repeat-TRP. As such, the sample size of the population was relatively small. Thus, larger sample sizes should be conducted to validate our results. Moreover, further studies are needed to estimate the developmental changes in arterial morphology in mid and long term studies *in vivo*.

In conclusion, VHFUBM is significantly beneficial in the detection of layer histopathological morphology and structure. Puncture as well as insertion of the sheath during transradial coronary could give rise to modified structures of the vessel wall. Increased RA trauma from repeat TRP might provoke more serious lesions to all layers of the vessel wall. Efforts of using smaller diameter guide catheters, improving procedural technique, and employing the use of pharmacological interventions may minimize radial wall injury.

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