Embolitic material generated by multiple aortic crossclamping: A perfusion model with human cadaveric aorta

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Background: Atherosclerosis of the ascending aorta and use of aortic crossclamping are risk factors for neurologic injury during cardiac surgery.

Objectives: Repeated aortic manipulation is part of the surgical approach to most cardiac operations. The aim of this study was to assess the amount and size of particulate matter that is dislodged from the aortic wall as a function of repeated aortic crossclamping.

Methods: In 10 subjects undergoing autopsy the aorta was dissected and mounted in a perfusion model. The ascending aorta was crossclamped and washed out 10 times, with the perfusate collected in aliquots (1 to 10). The aliquots were examined by computerized image processing, both macroscopically and under the microscope for calcified and cellular material.

Results: Aortic crossclamping produced substantial output of particulate matter. After repeated aortic crossclamping the number of particles decreased ($P = .012$) and approached the baseline for aliquots 6 to 10. The average particle diameter was 0.63 ± 0.03 mm, with a maximum of 4.74 mm. Similar variability in particle outputs were recorded microscopically, with findings of both calcified and cellular material. Nine of 10 aortas had calcifications seen during simple visual inspection.

Conclusions: The washouts of dislodge material at aortic crossclamping had embolic potential. During the initial aortic crossclamping procedures the amount of particles was substantial, both macroscopically and microscopically. On the microscopic scale noncalcified cellular debris represents a significant pool of embolic material. Repeated aortic crossclamping reduced the amount of particles. These findings question surgical techniques associated with repeated aortic crossclamping.

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Brain damage is a significant part of morbidity and mortality after cardiac surgery. The scenario of brain damage is complex and has two extremes, major stroke versus diffuse cognitive defects.¹ Atherosclerosis of the ascending aorta and aortic crossclamping (ACC) has been identified as major risk factors for perioperative stroke during cardiac surgery.^{2,3} Diffuse symptoms of brain damage occurs in the postoperative period and are often discussed in relation to the use of extracorporeal circulation, such as from fat microembolism of retrieved mediastinal blood,^{4,5} or in terms of immunological mechanisms.⁶ In addition, cerebral injury may be secondary to hypoperfusion and anesthetic management.⁷

Most types of cardiac surgery involve manipulation of the aorta by clamping.⁸ A number of techniques have been used to reduce the risk of stroke during cardiac operation,^{8,9} including echo visualization of the aorta.¹⁰ In coronary artery bypass grafting (CABG) on the nonbeating heart, two different methods are employed to

TABLE 1. Subject demographics, autopsy data, and aorta characteristics

Parameter	Mean $\pm$ SEM (%)	Range (min-max)
Age (years)	72.7 $\pm$ 3.8	51-84
Males (%)	60	
Clinical diagnosis		
Diabetes (%)	30	
Hypertension (%)	20	
Cardiac data at autopsy		
Previous myocardial infarction (%)	40	
Coronary disease (%)	90	
Valvular disease (%)	60	
Cause of death		
Acute myocardial infarction (%)	40	
Circulatory failure unspecific (%)	40	
Cardiac tamponade (%)	10	
Pneumonia (%)	10	
Specific aortic observations		
Aortic diameter (mm)	32.1 $\pm$ 1.4	25.5-38.2
Aortic media wall thickness (mm)	1.6 $\pm$ 0.1	1.1-2.2
Magnitude of calcification (0-4)	2.0 $\pm$ 0.3	0-3
Ulcerative calcifications (%)	60	

perform distal anastomoses, either cardioplegic arrest or the older method of fibrillating heart. The purpose of this study was to investigate the mechanism of injury caused by ACC. The focus was on repeated clamping, such as with the fibrillating heart method.

An experimental model was designed using human cadaveric aorta. The spectrum of dislodged material following ACC was evaluated using computerized image processing, both of macroscopic and microscopic particulate matter. The macro- versus microscopic approach was done with reference to possible mechanisms behind stroke and diffuse brain damage, respectively.

Materials and Methods

Subjects

The study was based on 10 subjects undergoing autopsy. The subjects all belonged to the high-risk group of atherosclerosis (Table 1). The subjects were selected at random from the daily program of autopsy during a 2-month study period. The experiments were performed within 72 hours after death. Prior to the investigation 2 subjects were tested for method evaluation. During the study an additional 2 subjects were excluded due to findings of aortic dissection and technical circumstances during the autopsy, respectively. The study was approved by the Umeå University ethical committee (Dnr 01-142).

Aortic Perfusion Model

The heart and lungs were removed en block according to standard autopsy procedure. The aorta was freed from surrounding connective tissue. The aorta was fixed to a foam-plastic workbench using needles through vessel branches. The major arterial branches were

Figure 1. The ACC perfusion model is schematically illustrated. The surgical situation at aortic declamping was simulated by a pressurized human cadaveric aorta and retrograde washouts. The crossclamp was released to fill a 50-mL test tube from the ascending aorta, followed by reoccluding. The procedure was repeated 10 times. Large side branches were occluded by ligatures and small arteries by clamps. The perfusion cannula was connected to a constant pressure of 60 mm Hg.

closed by ligatures, and the remaining vessels were closed using small surgical clamps.

Retrograde perfusion was implemented (Figure 1). A catheter (8/10.6 32, Polystan A/S, Værløse, Denmark) was positioned in the descending aorta and fixed with ligatures so that the perfusion tip was localized at the subclavian artery. The cannula was connected to a constant pressure device consisting of a 10-L infusion bag positioned at a hydrostatic level corresponding to 60 mm Hg pressure. The perfusion medium consisted of 9 g/L NaCl. The aorta was flushed with about 400 mL perfusion medium to remove debris, followed by a 50-mL sample of the perfusate representing the baseline prior to ACC (denoted as ACC 0). A standard 70-mm aortic crossclamp (Pilling Co, Fort Washington, Pa) was applied to the ascending aorta at a location that resembles a standard surgical procedure. This was typically about 2 cm proximal to the brachiocephalic trunk. To precisely repeat the crossclamping at the same location, the crossclamp jaws were fixed to the aortic adventitia with stitches. To gain the same occluding force of repeated clamp-

Figure 2. Typical deposits collected on a microscopic slide are shown. (a) Macroscopic view of a calcified particle at $\times 30$ magnification. A section is indicated for close-up microscopy in panel b. (b) Microscopic view of particle deposits at $\times 240$ magnification. The images were subject to gray-scale contrast enhancement.

ing, the clamp was closed to half of its full range in all experiments.

During constant pressure load, the clamp was momentarily opened for collection of a washout sample in a 50-mL test tube (Falcon, Sarstedt, Nümbrecht, Germany). The clamp was closed without loosening further perfusion medium and the aorta con-

stantly filled up with new medium from the infusion bag. This procedure was repeated 10 times (sample ACC 1 to 10). The entire output of perfusate was collected in this way and the recorded data therefore refer to all particulate matter from the manipulated aortic region. After ACC manipulation, the pathologist cut and opened the aortic arch to evaluate the type and grade of atherosclerotic

Figure 3. Geometric characteristics of calcified particles on the macroscopic scale are demonstrated. Deposits were collected from the ascending aorta following repeated ACC. A baseline recording prior to clamping is denoted by zero on the horizontal axis. The curves represent deposits produced at ACC 1 to 10. (a) Particle number, (b) particle average area, (c) particle shape factor, and (d) embolic load from multiplying the number of particles with particle area. Mean values $\pm$ SEM. Reference is made to Table 2 for statistical results.

intimal lesions. Atherosclerotic grading was according to a semi-quantitative scale: 0, normal; 1, mild; 2, moderate; 3, severe; and 4, "porcelain aorta" (Table 1).

Sample Processing

Each washout was centrifuged at 1500g for 10 minutes at 22°C. The supernatant was carefully aspirated, leaving the deposit in the test tube. Distilled water (50 mL) was added to the sample to lyse remaining erythrocytes and for washing purpose. The sample was recentrifuged and the supernatant was again aspirated. The collected material was fixed for 10 minutes at 22°C, by adding 5 mL of 10% formalin in phosphate buffer. The fixation was stopped by adding distilled water, followed by washing and recentrifugation. The material was stained for 10 minutes at 22°C with 20 μ L of cresyl violet for basophilic material,¹¹ followed by 2 washing and centrifugation cycles. The sample was collected in a plastic Pasteur pipette and deposited on an uncoated microscopic slide. The drop-let was spread out and left to dry at room temperature. When a sample contained high amounts of particulate matter, it was spread out on 2 separate microscopic slides. This was done to avoid severely condensed material that would interfere with the image analysis. With duplicate slides, which occurred in 20% of all

samples, the particulate matter from both slides was added together during image analysis. A typical deposit is shown in Figure 2.

Image Analysis of Particulate Matter

The microscopic slides were assessed using an image analyzer (Zeiss KS 300, version 3.0, Carl Zeiss Vision GmbH, Hallberg-moss, Germany). Each deposit was analyzed both macroscopically and in the microscope. For macroscopic evaluation the slide was positioned on a lighted stage and observed via a video camera (Sony DXC-101P, Tokyo, Japan). With this method the entire deposit from each clamp opening was scanned in 1 image, or from the summation of 2 recordings for duplicate slides. The recorded data on macroscopic level thus refer to both a quantitative and a qualitative analysis of the particulate matter.

For microscopic evaluation the samples were viewed in an inverted microscope (Olympus CK40-F200, Olympus Optical Company Ltd, Tokyo, Japan) equipped with a CDPlan $\times 10$ -FPL objective lens, and recorded using a black and white camera (C5405-01 Hamamatsu Photonics, Hamamatsu City, Japan). In this configuration 2 microscopic views were sampled at random from each slide. The microscopic data therefore represent a qualitative measure of the deposit.

TABLE 2. Characteristics of particle deposits

Parameter	ACC	Unit	Macroscopic A, calcified particles (mean $\pm$ SEM)	Within A, Wilcoxon P value	Between A-B, MWU P value
Particles					
ACC	average 1-5	n/mL	10.2 $\pm$ 3.9		NA
ACC	average 6-10	n/mL	2.96 $\pm$ 1.17	.012	NA
Slope	1-10	n/mL/clamp	-1.50 $\pm$ 0.65	.008	NA
Area					
ACC	average 1-5	mm ² or μ m ²	0.193 $\pm$ 0.063		<.001
ACC	average 6-10	mm ² or μ m ²	0.104 $\pm$ 0.033		.003
Slope	1-10	mm ² or μ m ² /clamp	-0.014 $\pm$ 0.010		
Shape factor					
ACC	average 1-5	—	0.586 $\pm$ 0.076		.029
ACC	average 6-10	—	0.479 $\pm$ 0.094		.010
Slope	1-10	-/clamp	-0.021 $\pm$ 0.013		
Particles $\times$ area					
ACC	average 1-5	$n \times$ mm ² or μ m ² /mL	1.91 $\pm$ 0.54		NA
ACC	average 6-10	$n \times$ mm ² or μ m ² /mL	0.480 $\pm$ 0.218	.037	NA
Slope	1-10	$n \times$ mm ² or μ m ² /mL/clamp	-0.275 $\pm$ 0.090	.013	NA

NA, Not applicable.

The average volume of the washout samples was 35.0 $\pm$ 0.9 mL (mean $\pm$ SEM). Due to technical circumstances a few washouts deviated in volume with a range from 15.0 to 51.5 mL. Due to this variation the amount of deposits was corrected for the sample volume and expressed as particles per milliliter. For the microscopic evaluation, the data were expressed per milliliter perfusate and per microscopic view.

Image Processing and Geometric Measurements

The images were processed by computerized technique to measure particle count, area, and shape factor. The image handling included: (1) contrast enhancement to full gray scale, (2) manual editing for technical artefacts, (3) 1-step pixel dilation followed by pixel erosion, and (4) geometric measurement from preset gray threshold levels.

On the macroscopic level the particulate matter was dense in contrast and evaluated by a single threshold setting for calcified particles. On the microscopic level, at which both calcified dense and cellular semitransparent particulate matter were detected, the image analysis was performed using 2 windows of gray scale attenuation, dense and soft spectrum, respectively. All threshold settings were constant throughout the study. The results were transferred to an Excel spreadsheet.

Statistical Analysis

The statistical evaluation contained ordinal, discrete, and numerical data. The geometric data (eg, area and shape factor measurements) showed normality. Mean values $\pm$ SEM are given throughout. However, because of the different nature of data only nonparametric statistics were used. For within-group differences Wilcoxon signed rank test was applied. Between-groups differences were evaluated by Kruskal-Wallis test and post hoc comparison by Mann-Whitney *U* test. Correlation matrices were tested using Spearman's rank coefficient. Slope values refer to linear regression versus clamp opening. A *P* value above .05 was considered nonsignificant.

Results

Demographics of Subjects

Demographic and autopsy data are summarized in Table 1. From a semiquantitative evaluation of calcification, 1 subject was without overt signs of calcification in the aorta. In the remaining 9 cases the aorta had atherosclerotic changes of variable magnitude and ulcerations. None of the subjects had porcelain characteristics (Table 1). There was no significant correlation between subject age and magnitude of aortic calcification.

Method Evaluation

We developed and tested a new method of studying the injuries caused by repeated ACC. The method gave reproducible results by which the accumulated deposits varied in number and characteristics with repeated ACC. There was a significant correlation between number of deposits and the magnitude of aortic wall calcification (*P* = .014). There was no correlation between subject age and number of deposits.

Macroscopic Particle Evaluation (Dense Spectrum)

The first-time clamping procedure resulted in a marked increase in observed calcified particles versus the baseline recording (Figure 3, *a*). With repeated ACC the number of deposits decreased. ACC 1 to 5 produced significantly larger amounts of particles compared to ACC 6 to 10 (Table 2, column A). This finding was also confirmed by a negative slope that was significantly different from zero (Table 2). There were no significant differences in the observed average area or shape factor of the calcified particles between ACC 1 to 5 compared with ACC 6 to 10 (Figures 3, *b* and *c*, Table 2). When the embolic particle load was calculated, by multiplying the area with particle numbers, there was

TABLE 2. Continued

Microscopic B, calcified particles (mean $\pm$ SEM)	Within B, Wilcoxon <i>P</i> value	Between B–C, MWU <i>P</i> value	Microscopic C, cellular and calcified (mean $\pm$ SEM)	Within C, Wilcoxon <i>P</i> value	Between A–C, MWU <i>P</i> value	Between A–B– C, Kruskal- Wallis, <i>P</i> value
1.18 $\pm$ 0.14		NA	4.64 $\pm$ 0.67		NA	NA
0.746 $\pm$ 0.076	.017	NA	2.86 $\pm$ 0.50	.028	NA	NA
–0.057 $\pm$ 0.024	.037		–0.315 $\pm$ 0.117	.013	NA	NA
190 $\pm$ 66			129 $\pm$ 39		<.001	.001
152 $\pm$ 37		.041	96.3 $\pm$ 30.7	.005	.003	.006
–6.95 $\pm$ 4.74			–6.50 $\pm$ 2.09	.005		
0.786 $\pm$ 0.025		.029	0.862 $\pm$ 0.015		<.001	.002
0.796 $\pm$ 0.024		.013	0.875 $\pm$ 0.013	.007	.001	.002
0.004 $\pm$ 0.002			0.003 $\pm$ 0.001	.005		
85.7 $\pm$ 43.1		NA	255 $\pm$ 117		NA	NA
42.5 $\pm$ 13.5	.005	NA	93.4 $\pm$ 31.3	.007	NA	NA
–9.73 $\pm$ 7.29	.013	NA	–34.3 $\pm$ 20.7	.007	NA	NA

again a significant difference between ACC 1 to 5 versus ACC 6 to 10 (Figure 3, *d*, Table 2). This variability produced a negative slope significantly different from zero (Table 2). The average diameter of recorded particles of all measurements was 0.63 ± 0.03 mm, with a spread from 0.22 to 4.74 mm (0.22 mm represents the technical resolution for the macroscopic measurements).

Microscopic Particle Evaluation (Dense Spectrum)

The average ACC 1 to 5 produced significantly larger amounts of microscopic dense particles (eg, calcified material) compared with ACC 6 to 10 (Figure 4, *a*, Table 2, column B). The slope became significantly negative (Table 2). There were no significant differences when comparing the average area or shape factor of the calcified particles between ACC 1 to 5 and ACC 6 to 10 (Figures 4, *b* and *c*, respectively, Table 2). The embolic particle load was calculated (particles $\times$ area) and demonstrated a significant reduction in particles between ACC 1 to 5 versus ACC 6 to 10, with a significant negative slope (Figure 4, *d*, Table 2). The average diameter of recorded microscopic particles of all measurements was 14.3 ± 0.6 μ m with a spread from 3.1 μ m to 633 μ m.

Microscopic Particle Evaluation (Soft Spectrum)

The average ACC 1 to 5 produced significantly larger amounts of microscopic particles (eg, spectrum sensitive to cells including dense calcified particles) compared with ACC 6 to 10 (Figure 5, *a*) and with a negative slope (Table 2, column C). There was a significant reduction in size of detached material with repeated ACC (Figure 5, *b*, Table 2). More circular deposits were observed as a function of

repeated ACC, and with an increased shape factor (Figure 5, *c*, Table 2). The calculated embolic load indicated a decrease in particulate matter with repeated ACC (Figure 5, *d*, Table 2). The average diameter of recorded particles of all measurements, and with the microscopic configuration, was 10.7 ± 0.4 μ m with a spread from 3.1 to 851 μ m.

Comparison between Calcified and Cellular Material

With geometric assessment of particles (eg, area and shape factor) it is possible to compare the 3 particle categories: macroscopic, microscopic, and cellular particles. The macroscopic method gave geometrically larger particles than what was technically measured under the microscope (Table 2). With the microscopic method the dense spectrum of calcified material was significantly larger in size compared with what was measured using the soft spectrum of cellular material. The shape factor analysis indicated that the larger macroscopic particles were more irregular in shape than the microscopic deposits. Further, the cellular material suggested more circular shape than their calcified counterparts (Table 2).

Because of the different recording methods between macroscopic and microscopic methods it was not meaningful to compare particle numbers between these groups. However, the two microscopic evaluation methods were possible to compare. Of particular interest was the embolic load from the product of particle- $\times$ -area. There was a significantly larger amount of embolic output of cellular material, comparing columns C versus B (Table 2). From a technical image-analysis perspective this was expected as the cellular measuring window also contained calcified ma-

Figure 4. Geometric characteristics of particles on the microscopic scale are shown, with the attenuation window set for dense calcified material. Deposits were collected from the ascending aorta following repeated ACC. A baseline recording prior to clamping is denoted by zero on the horizontal axis. The curves represent deposits produced at ACC 1 to 10. (a) Particle number, (b) particle average area, (c) particle shape factor, and (d) embolic load from multiplying the number of particles with particle area. Mean values $\pm$ SEM. Reference is made to Table 2 for statistical results.

terial. If the calcified material was subtracted from the data, leaving only cellular debris, it was found that the perfusate contained 169 ± 41 ($\text{n} \times \mu\text{m}^2/\text{mL}$ and microscopic view) of cellular embolic material. This was twice the amount of calcified particles, 85.7 ± 43.1 ($\text{n} \times \mu\text{m}^2/\text{mL}$ and microscopic view) (Table 2, columns B and C). However, this apparent difference was not statistically significant ($P = .070$).

Variability between Subjects

The data showed great variability between the cases. Although 9 of the 10 subjects had atherosclerotic changes in their aorta, only half of the cases produced major bursts of dislodged output (Figure 6). This was evaluated by calculation of linear regression. In 9 subjects the slope was negative, although in 4 of these only minor negativity was found (Figure 6). The patient without visual aortic calcifications belonged to the group that had a slope close to zero.

Discussion

Recent studies have demonstrated a link between the degree of atherosclerosis of the ascending aorta and neurologic damage after cardiac surgery.^{3,10,12} Further, perioperative complications in the central nervous system is known to be higher in older patients undergoing CABG.^{9,13} A number of new techniques have been used to reduce the risk of stroke after CABG, including single-clamp technique,⁹ “non-touch technique,” and various forms of off-pump surgery.¹⁴ However, a majority of surgical techniques involve some manipulation of the aorta.² The use of aortic crossclamp is known to release particulate emboli into the systemic circulation.¹³ Microscopic analysis has shown clamp-induced trauma of the aortic wall.¹⁵ The use of intra-aortic filtration has been proposed to reduce adverse neurologic events after cardiac surgery.¹⁶

Before the era of cardioplegic arrest, the method of choice for CABG was by transient fibrillation of the heart.¹⁷

Figure 5. Geometric characteristics of particles on the microscopic scale are shown, with the attenuation window at soft spectrum to include cellular material. Deposits were collected from the ascending aorta following repeated ACC. A baseline recording prior to clamping is denoted by zero on the horizontal axis. The curves represent deposits produced at ACC 1 to 10. (a) Particle number, (b) particle average area, (c) particle shape factor, and (d) embolic load from multiplying the number of particles with particle area. Mean values $\pm$ SEM. Reference is made to Table 2 for statistical results.

With this technique the aorta was clamped repeatedly, for each anastomosis. Even though this method may be considered old-fashioned, it is still in use at several clinics around the world.¹⁸ A parallel scenario is the manipulation from side-biting aortic clamps. In this study we simulated the surgical methods of fibrillating heart or other forms of repeated clamping. The crossclamp was applied repeatedly and in the same location on the aorta, and the dislodged material was collected and geometrically analyzed. The hypothesis was that the magnitude of dislodged particles would diminish with repeated clamping. This assumption was here confirmed, although the baseline was reached after several clamp maneuvers.

Aortic particles that cause stroke are thought to be geometrically large, such as detached calcified plaques. These particles are best analyzed on the macroscopic level. We found an average particle area of macroscopically detected material of about 0.2 mm². The average diameter was 0.6 mm, with maximum of 4.7 mm. Moreover, it was found that

the macroscopic material was noncircular with a shape factor of about 0.6 (circular shape equals 1 and linear shape 0). These data can be compared with known diameters of embolic material in occluded cerebral arteries (Willis circle) of about 5 mm.¹⁹ Our data corroborate histological observations from ongoing studies using intra-aortic filters, in which the diameter of captured particles ranged from 0.1 to 6.0 mm.¹³

Although the macroscopic material seems to be the most interesting with respect to stroke, cardiac surgery is also known to cause diffuse defects in neuropsychology and cognition.¹ This may in part reflect small-artery brain emboli.²⁰ It has been speculated that pericardial suction blood contains liquid fat that occludes brain capillaries' blood flow function.^{4,5} However, this study suggests an additional source of microscopic emboli from ACC. We investigated microscopic particles from two perspectives, both calcified and cellular material. The distinction between these particles was created by gray-scale attenuation at image analysis.

Figure 6. Characteristics of individual subjects on the macroscopic scale are demonstrated, using linear regression analysis of particle numbers produced at ACC 1 to 10. Deposits were collected from the ascending aorta following repeated ACC. Five of the 10 subjects produced substantial output of particles, as shown. In only 1 of the 10 subjects was the regression analysis slope positive.

Dense opaque material was calcified microparticles. Cellular material was measured using a softer threshold spectrum to expose semitransparent material. Red blood cells that contaminated the samples were lysed by water prior to the analysis, and the measured material was noncalcified cellular debris from the aortic wall. The average area of these particles was approximately 100 to 200 μm^2 , with measured diameters of about 10 to 15 μm for calcified and cellular material, respectively. The particles were more circular in shape than the large-size macroscopic deposits. The capillary diameter is known to be about 5 μm ,²¹ which suggests embolic potential.

We found that the magnitude of dislodged particles differed substantially between subjects. In this study only 5 of the 10 subjects produced large amounts of debris. There was a positive correlation between the amount of particles and the visual appearance of aortic atherosclerosis. Nine of the 10 cases had visible atherosclerosis, but in only half of these were severe amounts of particles dislodged at clamping.

Our study was limited by the use of cadaveric aorta. It is reasonable to expect that some postmortem changes may affect the results in different ways. Furthermore, the conditions at autopsy may not truly represent the surgical conditions in the operating room. The limited number of observations must also be considered. Our model employed retrograde washout, which is a simplification although it resembles the surgical situation at aortic declamping. However, the data appeared consistent, and image analysis revealed significant trends with repeated ACC, a fact that suggests a useful model in surgical science. The clinical impact of our findings can only be speculated upon as a question that relates to the complexity of flow dynamics and vascular anatomy.

In conclusion, we designed an experimental model with cadaveric aorta to analyze injury caused by crossclamping. Our main focus was on surgical methods using repeated ACC, such as with the fibrillating heart technique. Repeated crossclamping reduced the amount of dislodged particulate matter. However, the output of material was profound during the initial series of clamp maneuvers, a finding that questions repeated ACC in surgery.

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