SUPPLEMENTAL MATERIAL
Supplemental Methods

Perceval sutureless aortic valve bioprosthesis

The biologic component consists of glutaraldehyde-fixed bovine pericardium treated with homocysteic acid and the stent is made of an elastic nickel-titanium alloy covered by Carbofilm (LivaNova). The design features one proximal and one distal ring segment and nine vertical struts designed to support the valve and allow the prosthesis to anchor to the aortic root and the sinus of Valsalva. The stent supports the valve and holds it in place without the need for suturing. To aid the positioning of the prosthesis into the aortic annulus, the inflow ring has three loops through which temporary guiding sutures are passed. At the back table before implantation, the valve is collapsed with a device-specific system provided by the manufacturer and after temporary deformation the valve can return to its original shape owing to elastic alloy design.

Sutureless surgical aortic valve replacement

A transverse aortotomy approximately 3.5 cm above the aortic annulus was performed. After removal of the native valve leaflets, complete decalcification of the annulus was performed. Product-specific seizers were used to estimate annular size. Three guiding sutures were placed at the nadir of each valve sinus and passed through the corresponding loops in the inflow ring of the valve prosthesis. At the back table, the prosthesis was collapsed and loaded onto the delivery device. The valve was released at the level of the aortic annulus, followed by dilation of the inflow ring segment with a specifically designed balloon catheter at 4 atm for 30 s. The guiding sutures were removed and the aortotomy closed. After weaning from cardiopulmonary bypass, intraoperative transesophageal echocardiography was performed to confirm correct positioning of the prosthesis.
**Cardiac computed tomography data acquisition**

All patients were informed to avoid nicotine and caffeine 4 hours prior to the cardiac CT examination. For patients without any contraindications and with a systolic blood pressure >110 mmHg, oral metoprolol (50 mg if heart rate 60-65 bpm, 100 mg if heart rate >65 bpm) was administered 1 hour prior to scheduled CT examination. A 16-gauge peripheral venous catheter was inserted into the antecubital vein. ECG leads were placed midclavicular and lateral.

All patients were scanned by using a dual source 2x64 row multidetector computed tomograph (Siemens Somatom Definition Flash, Siemens Healthcare, Forchheim, Germany). Scanning parameters were as follows: retrospective ECG gating, 100-120 kVp, automatic dose modulation (CARE Dose), full dose R-R scanning (no ECG mA modulation), quality reference 370 mAs, 64 x 0.6 mm detector collimation, 0.28 seconds rotation time (75 ms temporal resolution). In order to reduce the risk of arrhythmia during scanning the iso-osmolar intravenous contrast medium (CM) iodixanol 320 mg iodine per mL (Visipaque, GE Healthcare, Stockholm, Sweden) was used. The intravenous CM was injected with a fixed injection time of 8 seconds and a dose of 200 mg iodine/kg body weight. This resulted in a dose variation of 31-50 ml with an injection rate of 3.9-6.3 ml per second. For patients examined with 120 kVp, another 20% was added to the calculated individual CM dose, in order to compensate for the lower level of x-ray attenuation of the CM molecule at 120 kVp compared to 100 kVp. This resulted in a maximum CM dose of 60 ml. Delay time was defined by using the test bolus technique: 10 ml of iodixanol 320 mg iodine/ml was injected at the same individual injection rate calculated for the cardiac CT examination. Monitoring scans were obtained every second. A delay of 2 seconds was added to the time to peak automatically calculated by the CT scanner analysis program from a 10 mm region of interest placed in the ascending aorta.

All acquired CT data was reconstructed to 0.75 mm slice thickness with an increment of 0.4 mm at 5% intervals (20 phases) throughout the R-R interval and at best systolic and diastolic phase.
All reconstructed image data was transferred to a dedicated workstation (Advantage Workstation, ADW 4.6, GE Healthcare, Milwaukee, USA) for post-processing and time resolved volume rendering (VR). In order to simplify the transformation of the time-resolved image data set into dynamic VR reconstructions, four different 3D presets were created, corresponding to different levels of intravenous CM enhancement, measured as Hounsfield units (HU), in the ascending aorta. The 5% acquisition data set was used for the creation of time-resolved 4D VR. A 2-image 3D VR was made from the best systolic and diastolic phase (one en face VR image at maximal leaflet opening and one in diastole).

**Cardiac computed tomography analysis**

Cardiac CT data analysis was performed using the syngo.via software (Siemens Healthcare) on a PACS workstation. The optimal image display setting for leaflet visualization was chosen on an individual basis, but in general at a window width of 800–1200 Hounsfield units (HU) and a level of 200–300 HU.

In the presence of HALT, the maximum thickness of the cusp was measured in mid-diastole, using a 1-mm MPR perpendicular to the aortic annulus and to the cusp to be analyzed. The prosthetic valve leaflets were defined according to their location relative to the sinus of Valsalva: right cusp, left cusp, and non-coronary cusp and the number of affected cusps was noted.

During subsequent separate reading sessions, the two readers performed additional analyses of leaflet motion, with access only to three-dimensional (3D) volume-rendered (VR) images of the aortic-valve bioprosthesis, blinded to findings of previous MPR analyses. First, they had access to two 3D VR en face images of the aortic valve prosthesis: one image in diastole and one image at maximal leaflet opening, similar to analyses previously described. In a final reading, assessment of leaflet motion was based on time-resolved VR (4D VR) images.
Supplemental Results

Validation of computed tomography evaluation methods

When comparing two independent readers’ assessment of RLM, based on time-resolved MPR images of 46 patients, there was 100% agreement (Cohen’s kappa 1.0). Interobserver agreement regarding the presence of HALT, based on still MPR images, was almost complete (overall agreement 96%, Cohen’s kappa 0.91, 95% CI 0.78-1.0).

Evaluation of RLM based on 3D VR images alone correlated with findings based on thorough assessment of MPR, but interobserver variability was greater. For assessment of two VR en face images of the bioprosthesis (one in diastole and one at maximal leaflet opening; Figure 7) the proportion of overall agreement with MPR analysis was 85% (Cohen's kappa 0.68, 95% CI 0.47-0.89). For assessment of leaflet motion based on time-resolved VR (4D VR, Supplementary video 4-7) images overall agreement with MPR analysis was 89% (Cohen's kappa 0.76, 95% CI 0.57-0.95). Interobserver agreement was moderate and identical for the two methods of assessment based on VR images alone (overall agreement 76%, Cohen's kappa 0.54, 95% CI 0.33-0.75). No cases of RLM were missed when evaluating VR images alone, but there were false positive cases as compared with MPR assessments. We found 3 false positive cases for diastolic vs. systolic VR images, and 3 false positive cases for 4D VR images.
<table>
<thead>
<tr>
<th>Prosthesis size</th>
<th>Total population (n=47)</th>
<th>No HALT (n=29)</th>
<th>HALT (n=18)</th>
<th>Normal leaflet motion (n=33)</th>
<th>RLM (n=13)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>4/47 (9%)</td>
<td>4/4 (100%)</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.04 (0.66)</td>
<td>2.04 (0.66)</td>
<td>2.04 (0.66)</td>
<td>2.04 (0.66)</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Medium</td>
<td>18/47 (38%)</td>
<td>11/18 (61%)</td>
<td>7/18 (39%)</td>
<td>14/18 (78%)</td>
<td>4/18 (22%)</td>
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</tr>
<tr>
<td></td>
<td>1.85 (0.37)</td>
<td>1.95 (0.35)</td>
<td>1.69 (0.36)</td>
<td>1.93 (0.36)</td>
<td>1.56 (0.25)</td>
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<td>Large</td>
<td>20/47 (43%)</td>
<td>11/20 (55%)</td>
<td>9/20 (45%)</td>
<td>12/19 (63%)</td>
<td>7/19 (37%)</td>
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<tr>
<td></td>
<td>2.27 (0.34)</td>
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<td>2.04 (0.24)</td>
<td>2.37 (0.36)</td>
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<tr>
<td>Extra large</td>
<td>5/47 (11%)</td>
<td>3/5 (60%)</td>
<td>2/5 (40%)</td>
<td>3/5 (60%)</td>
<td>2/5 (40%)</td>
<td>0.25</td>
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<td></td>
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<td>2.73 (0.32)</td>
<td>2.13 (0.52)</td>
<td>2.73 (0.32)</td>
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Implanted prosthesis size and prosthetic valve opening area in relation to HALT and leaflet motion in 47 patients who underwent computed tomography at different time points after surgical aortic valve replacement with the Perceval sutureless aortic valve bioprosthesis. Data are n (%) and mean aortic valve area (standard deviation) in cm². HALT = hypo-attenuated leaflet thickening; RLM = reduced valve leaflet motion.
Figure S1. Perceval Sutureless Aortic Valve Bioprosthesis (LivaNova, Milan, Italy).
Figure S2. Cardiac Computed Tomography Multiplanar Reformatted Reconstructions of a Perceval Sutureless Aortic Valve Bioprosthesis in Mid-diastole.

The non-coronary cusp (panel A) was normal, with no signs of hypo-attenuated leaflet thickening. The left cusp (panel B) was markedly thickened with hypo-attenuated leaflet thickening. The maximum leaflet thickness was 5 mm (panel C). The three-valve leaflets are shown simultaneously; two of them normal and the left cusp with hypo-attenuated leaflet thickening (panel D).
Figure S3. Multiplanar Reformatted Reconstructions for Evaluation of Leaflet Motion in a Perceval Sutureless Aortic Valve Bioprosthesis. Top panels show images in diastole and bottom panels show images of maximum leaflet opening in systole. Images to the left show the normal right cusp (white circle) in diastole (panel A) and fully open in systole (panel B). Images to the right show the non-coronary cusp (dashed circle) of the same patient in diastole (panel C) and with reduced leaflet opening in systole (panel D). Hypo-attenuated leaflet thickening of the non-coronary cusp was also present. Dynamic images are presented in Videos S1A–D, 2A–B, 3A–D.
Figure S4. 3D Volume-rendered en face Images of the Perceval Sutureless Aortic Valve Bioprosthesis. Top panels show images in diastole and bottom panels show images in systole. Images to the left show a normal bioprosthesis in diastole (panel A) and in systole (panel B). To the right, a bioprosthesis with reduced motion of the right cusp (white arrow) is shown in diastole (panel C) and in systole (panel D). Dynamic 4D volume-rendered images are presented in Videos S4–S7).
Video Legends:

Videos S1A-D: Cardiac computed tomography (CT) dynamic multiplanar reformatted reconstructions (MPR) of a Perceval sutureless aortic valve bioprosthesis, with normal motion of all three leaflets. Preferred program for viewing: VLC Media Player. **S1A)** Images reconstructed in the opening plane of the valve, throughout the cardiac cycle, showing normal motion of all three leaflets. **S1B)** Images reconstructed perpendicularly to the right cusp, throughout the cardiac cycle, showing normal motion of the right cusp (located to the left in the dynamic images). **S1C)** Images reconstructed perpendicularly to the left cusp, throughout the cardiac cycle, showing normal motion of the left cusp (located to the left in the dynamic images). **S1D)** Images reconstructed perpendicularly to the non-coronary cusp, throughout the cardiac cycle, showing normal motion of the non-coronary cusp (located to the right in the dynamic images).

Videos S2A-B: Cardiac computed tomography (CT) dynamic multiplanar reformatted reconstructions (MPR) of a Perceval sutureless aortic valve bioprosthesis, with reduced leaflet motion of one cusp. Preferred program for viewing: VLC Media Player. **S2A)** Images reconstructed in the opening plane of the valve, throughout the cardiac cycle, showing reduced leaflet motion of the left cusp and normal leaflet motion of the other two cusps. **S2B)** Images reconstructed perpendicularly to the left cusp, throughout the cardiac cycle, showing markedly reduced motion of the left cusp (located to the right in the dynamic images). Hypo-attenuated leaflet thickening of the left cusp is present.

Videos 3A-D: Cardiac computed tomography (CT) dynamic multiplanar reformatted reconstructions (MPR) of a Perceval sutureless aortic valve bioprosthesis, with reduced leaflet motion of two cusps. Preferred program for viewing: VLC Media Player. **S3A)** Images reconstructed in the opening plane of the valve, throughout the cardiac cycle, showing reduced leaflet motion of the left and right cusps and normal leaflet motion of the non-coronary cusp.
**S3B)** Images reconstructed perpendicularly to the left cusp, throughout the cardiac cycle, showing reduced motion of the left cusp (located to the right in the dynamic images). Hypo-attenuated leaflet thickening of the left cusp is present. **S3C)** Images reconstructed perpendicularly to the right cusp, throughout the cardiac cycle, showing reduced motion of the right cusp (located to the right in the dynamic images). Hypo-attenuated leaflet thickening of the right cusp is present, involving only the basal parts of the leaflet. **S3D)** Images reconstructed perpendicularly to the non-coronary cusp, throughout the cardiac cycle, showing normal motion of the non-coronary cusp (located to the left in the dynamic images).

**Videos S4-7:** Cardiac computed tomography (CT) time-resolved volume rendered (4D VR) en face images of four different Perceval sutureless aortic valve bioprostheses. Preferred program for viewing: VLC Media Player.

**Video S4:** Aortic valve bioprosthesis with normal motion of all three leaflets, throughout the cardiac cycle.

**Video S5:** Aortic valve bioprosthesis with normal motion of all three leaflets, throughout the cardiac cycle.

**Video S6:** Aortic valve bioprosthesis with reduced leaflet motion of the left cusp.

**Video S7:** Aortic valve bioprosthesis with reduced leaflet motion of the right cusp. Reduced motion is also present in a portion of the left cusp, but not in the entire cusp (which is more reliably evaluated using multiplanar reformatted images).