New ultrasonic radiation reduces cerebral emboli during extracorporeal circulation

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Abstract

Objective: Cardiac surgery is associated with intraoperative cerebral emboli, which can result in postoperative neurological complications. A new ultrasonic transducer (EmBlocker™) can be positioned on the ascending aorta and activation of the EmBlocker™ is expected to divert emboli to the descending aorta, thereby decreasing emboli in the cerebral arteries. In this preliminary animal study, safety and efficiency of this technology were examined. Methods: In 14 pigs (±70 kg), a median sternotomy was performed and the EmBlocker™ was positioned on the ascending aorta and the EmBlocker™ was alternately activated with high power for emboli injections and low power for air emboli injections. Transcranial Doppler (TCD) was used to analyse middle cerebral artery blood flow for occurrence of embolic signals, which were manually counted offline. Results: Histopathology revealed no difference between control and sonicated tissue. There is a rise in temperature during EmBlocker™ activation, but in all measured tissues it was within limits; less than 42°C for 2 min in the aorta wall directly under the EmBlocker™. Use of the EmBlocker™ significantly reduced emboli in the cerebral arteries in an animal model; air emboli (size 500 μm–700 μm) were introduced in the proximal ascending aorta and the EmBlocker™ was alternately activated with high power for emboli injections and low power for air emboli injections. Transcranial Doppler (TCD) was used to analyse middle cerebral artery blood flow for occurrence of embolic signals, which were manually counted offline. Results: Histopathology revealed no difference between control and sonicated tissue. There is a rise in temperature during EmBlocker™ activation, but in all measured tissues it was within limits; less than 42°C for 2 min in the aorta wall directly under the EmBlocker™. Use of the EmBlocker™ significantly reduced emboli in the cerebral arteries in an animal model; air emboli with 65% (left) and 69% (right) and solid emboli with 49% (left) and 50% (right). Conclusions: The new ultrasound technology can safely be applied and is capable of reducing emboli in the cerebral arteries during extracorporeal circulation. Use of the EmBlocker™ in cardiac surgery bears the potential to lower the risk of postoperative neurological complications. Clinical feasibility studies are in progress.

Keywords: Cerebral embol; Transcranial Doppler; Cardiac surgery; Ultrasound

1. Introduction

Several interventions in cardiac surgery are associated with the occurrence of intraoperative cerebral emboli [1–4]. Transcranial Doppler (TCD) is a non-invasive method which permits intraoperative visualisation of these cerebral emboli. Using TCD, different causes of cerebral emboli have already been identified such as: cannulation, cardioplegia needle incision, cross-clamp placement, start and stop of cardiopulmonary bypass (CPB) and de-clamping [2]. The composition of the emboli can be either gaseous or solid (fat or calcified plaque) and is mostly related to the kind of intervention. Irrespective of the type of emboli composition, a correlation between the amount of emboli and neurological complications has been found [2,4]. The occurrence of neurological complications after cardiac surgery varies from 2% to 8% for stroke and 5% to 43% for cognitive decline [1–5]. By reducing the number of cerebral emboli, the risk of neurological complications will most likely be reduced. In this study, a new ultrasonic device is examined that has the potential to reduce the number of cerebral emboli. An ultrasonic transducer (EmBlocker™) is positioned on the distal ascending aorta and by activating the ultrasonic power emboli are expected to divert into the aorta descendens and thereby reducing the emboli entering the innominate
artery and left common carotid artery. The aim of this preliminary animal study was to investigate the safety and the efficiency of this new ultrasonic technology.

2. Materials and methods

Three different protocols were part of this study. In one animal, the temperature was measured at different locations during activation of the EmBlocker™. In six animals, a safety study was performed. Following EmBlocker™ activation, the animals were kept for 1 week. After this week, tissues were collected for histopathology. During follow-up blood samples were taken to examine the hematology. In the third group (n = 7), the efficiency of the EmBlocker™ was assessed using TCD.

All animals received humane care in compliance with the ‘Guide for the care and use of laboratory animals’ of the National Institutes of Health. The study was approved by the local animal ethics committee.

2.1. The EmBlocker™

The new ultrasonic device, the EmBlocker™ (Neurosonix, Rehovot, Israel), consists of a round 2.2 MHz transducer with a diameter of 36 mm which is developed for placement on the aorta ascendens at the level of the bifurcation of the aorta and the innominate artery after thoracotomy as shown in Figs. 1 and 2. The EmBlocker™ was kept in position with an ESTECH stabilizer (ESTECH, San Ramon, United States of America). The mechanism of the EmBlocker™ is based on the principle that an object with different acoustic impedance (density multiplied by sound speed) than its surroundings partly reflects and partly absorbs ultrasound energy. The acoustical energy which is absorbed by an object is also called the acoustic radiation force, and this energy is able to move an object or to change the direction of its path [6,7]. The acoustic radiation force on an object depends on the extent of the difference between the acoustic impedance of the object and its surroundings; the greater the difference in acoustic impedance is, the higher the acoustical force on the object will be. Due to the fact that there is hardly an acoustic difference between red and white blood cells and its surroundings, the ultrasonic radiation power will not affect these cells. The acoustic impedance difference between gaseous emboli and its surroundings exceeds the acoustic impedance difference between solid emboli and its surrounding [8]. For this matter a lower ultrasonic energy level of the EmBlocker™ can be sufficient to achieve the same acoustic radiation force on gaseous emboli as a higher ultrasonic level of the EmBlocker™ on solid emboli. Due to this knowledge and in-vitro testings, an intensity level of the EmBlocker™ of 0.5 W/cm² is chosen to divert gaseous emboli and an intensity level of the EmBlocker™ of 1.5 W/cm² is chosen to divert solid emboli.

2.2. Animal preparation

2.2.1. Temperature

One pig (70 kg) was subcutaneously administrated with xylazine, diazepam and ketamine HCl 30 min prior to intubation. An endotracheal intubation was conducted and was followed by mechanical ventilation. Maintenance of general anesthesia was performed with isoflurane during the whole procedure. After administration of the anesthesia, a mid sternotomy was performed. The EmBlocker™ transducer was placed at the level of the bifurcation of the aorta and the innominate artery (Fig. 2). Temperature sensors were placed in the beam of the transducer in the esophagus, trachea, under aorta and on aorta. Also, temperature sensors were positioned in control areas on the aorta and in the esophagus outside the ultrasonic beam of the transducer. All animals were exposed twice to the pretended clinical duration of sonication: 120 s of 1.5 W/cm² intensity of the EmBlocker™ and 600 s of 0.5 W/cm² intensity of the EmBlocker™. This protocol was repeated four times in the same animal.

2.2.2. Histopathology and hematology

Six female domestic pigs approximately 70 kg (about 4 months old) were subcutaneously administrated with xylazine, diazepam and ketamine HCl 30 min prior to intubation. After the baseline blood sample was taken, endotracheal intubation was conducted and was followed by mechanical ventilation. Maintenance of general anesthesia
was performed with isoflurane during the whole procedure. After administration of the anesthesia, a mid sternotomy was performed using a sterile technique. The EmBlocker™ transducer was placed at the level of the bifurcation of the aorta and the innominate artery. The position of the transducer was documented to allow identification of the sonicated tissues. Then all animals were exposed to a combination of eight periods of 120 s of 1.5 W/cm² intensity of the EmBlocker™ and one period of 20 min of 0.5 W/cm² intensity of the EmBlocker™ (90 s on and 30 s off). After sonication, the chest was surgically closed in layers and blood samples were taken. Each animal recovered from anesthesia in the operating room, and then was transported to the step-down unit. Each animal received antibiotic (cefazolin 30 mg/kg by i.v., a single dose was given at the conclusion of the procedure and bytril 1 ml/20 kg intramuscular injection — once a day for 4 days) and analgesic agents (morphine single dose was given at the conclusion of the procedure and depirone for 3 days) in the postoperative period. After each clinical stage the animals were observed daily for signs of distress that would indicate the need for administration of analgesics. Seventy-two hours post surgery, a blood sample was taken. At day 7 postoperatively, all animals were sacrificed. Blood samples were taken prior to euthanasia. Post euthanasia, a mid-re-sternotomy was performed. Tissues were taken from sonicated and non-sonicated areas of the aorta, the innominate artery (or the junction of the innominate artery and aorta as a sonicated tissue), the trachea, the esophagus and the vagus nerve. The tissue samples were divided in two sections: back and front, whereby the front section is chosen as that which first comes into contact with the ultrasound beam. The tissues were fixed in formalin for at least 1 week and prepared routinely for histopathology.

Blood samples were analysed for general chemistry, free hemoglobin and hematology variables. Blood samples were taken from the animal at four different points of time: at baseline (prior to any surgical intervention); post closure; 72 h after surgery; and before sacrifice (after 1 week).

2.2.3. Efficiency

Seven pigs (75 ± 20 kg) were premedicated with 0.07 ml/kg azaperone (stresnil 40 mg/ml) intramuscularly. Anesthesia was induced with a mask of 1.5% isoflurane and with help of xylacaine spray (10%). The pigs were orally intubated. Anesthesia was maintained with a mixture of O2 and isoflurane (1.5%). After administration of analgesic buprenorphine (i.v. bolus 0.01 mg/kg) and muscle relaxant suxamethonium (i.v. bolus 0.1 mg/kg), a mid sternotomy was performed. Heparin was administered (bolus 200 IU/kg i.v.). After the activated clotting time (ACT) reached 300, an extracorporeal circulation with central cannulation was installed. ACT was kept above 480 s during the experiment. After the extracorporeal circulation had been started anesthesia was maintained with a propofol i.v. drip. Monitoring included ECG, blood pressure, oxygen saturation and capnography.

A cardioplegia needle was placed in the aortic root distal from the arterial cannula, which was used for emboli injections. Polystyrene DVB particles (Duke Scientific Corp) (1200,500—750 μm) were dissolved in a 10 ml syringe filled with a mixture (1:33) of serum and ringer’s lactate. Air emboli were created by mixing 1 ml of air with 9 ml of blood. Immediately after the mixture, the 10 ml was injected. Each kind of injection was performed 10 times; 5 times without EmBlocker™ activation (control) and 5 times with activation of the EmBlocker™ (sonicated). The applied power of the EmBlocker™ transducer during air emboli injections 0.5 W/cm² and for solid emboli injections is 1.5 W/cm². At the end of the experiment, all animals were sacrificed through a pentobarbital overdose (bolus 80 mg/kg i.v.).

For cerebral monitoring, two transcranial transducers (Embodop DWL, Singen, Germany) were placed on each eye of the pig. Through the opening of orbita of the eye, the ultrasound could pass the skull and penetrate into the white and gray matter of the brain and reach the cerebral blood vessels. A cerebral blood flow velocity spectrum from an intracranial artery could be obtained. Transcranial Doppler recordings were saved and analyzed offline. One observer manually counted the number of emboli according to the golden standard [9].

Statistical comparison (mixed model, two-tailed comparisons, n = 7) was performed to compare emboli counts between On and Off stages, while taking into account the fact that all animals are different and that each animal provides a number of correlated replicates. Statistical analyses were conducted with SAS/STATA® statistical software. The number of cerebral emboli was averaged over all replicated emboli injections for each type of emboli and for left and right measurements in all animals and presented as mean and standard deviation.

3. Results

In the safety study, all animals survived the operation, showed a course of steady clinical improvement, gained weight as planned and reached their target sacrifice date in good health and without any signs of illness or adverse events.

3.1. Temperature

In Table 1, the temperature values are shown from the esophagus, the trachea, under the aorta, on the aorta, control aorta and control esophagus at two time intervals during two different power settings of the EmBlocker™. The high power application (1.5 W/cm²) results in the highest temperature (41.9 °C) under the aorta after 2 min. At the low power application (0.5 W/cm²), the highest temperature measured (38.1 °C) was observed under the aorta after 10 min. All sonicated areas showed a rise in temperature during high and low power EmBlocker™ activation. The temperatures in the control areas, outside the ultrasound beam, remained constant during EmBlocker™ activation.

3.2. Histopathology

Only the atypical findings of the histopathology results are schematically given in Table 2. In five animals abnormalities in tissue form the aorta were determined. In four of these animals the abnormalities were established in control as in
sonicated tissue. In one animal just the sonicated aorta tissue demonstrated abnormalities. In two animals an inflammation in tissue of the trachea was determined. These findings were determined in one of these two animals in both control and sonicated tissue and in the other animal just in the sonicated tissue.

3.3. Hematology

The analyzed white blood cells (WBC), free hemoglobin (free HB) and the creatine phosphokinase (CPK) are shown in Table 3 at four periods in time (baseline, post closure, 72 h after thoracotomy and before sacrifice). The white blood cells are within normal limits during the entire follow-up period, but show a significant reduction between baseline and post closure. The free hemoglobin values are above the normal values at baseline, post closure and 72 h after thoracotomy. In the period just before sacrifice, the free HB is within range. Also, the CPK values are only within normal limits just before sacrifice and at all three other time points they are above the normal values. Although the first three values are higher than normal, there is a significant reduction immediately after the baseline values were obtained. Just before sacrifice, the CPK values significantly reduced to values within the normal range.

3.4. Efficiency

In all animals, both left and right velocity spectra of the cerebral arteries could be visualised with TCD. The quality of the recorded signals in each animal was suitable for the counting purposes. In Table 4, the number of cerebral emboli for the left and the right cerebral arteries are shown for solid as well as air injections with and without activation of the EmBlocker™. The number of emboli is significantly reduced by use of the EmBlocker™. In the case of air emboli injections, the number of emboli in the left middle cerebral artery is reduced from 155 emboli to 65 emboli and in the right middle cerebral artery from 182 emboli to 90 emboli, resulting in a reduction of, respectively, 58% and 51%. The activation of the EmBlocker™ reduced the number of solid emboli with 42% from 67 emboli to 39 emboli in the left middle cerebral artery and with 44% from 83 emboli to 46 emboli in the right middle cerebral artery.

4. Discussion

4.1. Temperature

The absolute temperature in different regions of the body was examined in one animal. As stated by Goldstein et al.

### Table 1

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Sonicated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Esophagus</td>
<td>Trachea</td>
</tr>
<tr>
<td>High power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>35.3</td>
<td>35.6</td>
</tr>
<tr>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>60</td>
<td>36.7</td>
<td>38.3</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>120</td>
<td>37.5</td>
<td>39.1</td>
</tr>
<tr>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

In the aorta and the esophagus also temperature measurements were performed outside the ultrasonic beam (control). At high power level the EmBlocker™ was activated up to 120 s, and at the low power level the activation lasted up to 600 s. Both time periods are twice the pretended clinically time period.

### Table 2

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aorta</td>
<td>Aorta</td>
</tr>
<tr>
<td>Remark</td>
<td>On the external surface of the adventitia is a focus of vascularised, inflamed granulation tissue.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Aorta</td>
<td>Aorta</td>
</tr>
<tr>
<td>Remark</td>
<td>On the external, adventitial surface is a thick layer of heavily inflamed vascularised granulation tissue including clumps of fibrin.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Aorta</td>
<td>Aorta</td>
</tr>
<tr>
<td>Remark</td>
<td>In the adventitial adipose tissue is a large area of heavily inflamed vascularised granulation tissue that includes fibrin on the surface and histiocytic giant cells.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Trachea</td>
<td>Trachea</td>
</tr>
<tr>
<td>Remark</td>
<td>The lamina propria is mildly expanded by acute and chronic inflammatory cells indicating a mild acute or chronic bronchitis.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Aorta</td>
<td>Aorta</td>
</tr>
<tr>
<td>Remark</td>
<td>On the external surface of the adventitia is a focus of vascularised, inflamed granulation tissue.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sonicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>No abnormalities</td>
<td>Aorta</td>
</tr>
<tr>
<td>Remark</td>
<td>The adventitia is mildly vascularised and mildly fibrotic but un inflamed.</td>
<td></td>
</tr>
</tbody>
</table>

The first column indicates the animal and the second and third column indicate if there was an abnormality found in the control and/or sonicated tissue. If the remark is shown in both second and third column, the abnormality was found in both control and sonicated tissue.
the 0.5 W/cm² energy level of the EmBlocker™ produces less heat than the 1.5 W/cm² energy level and can therefore be used for a longer time period. The high power application cannot be replaced clinically by the low power application because the high energy level is required to divert solid emboli. We can conclude that the inflammation and the tracheitis are not caused by sonication. Coagulation necrosis, which is considered to be a marker of damage caused by ultrasound induced hyperthermia, was not observed in any of the tissue samples.

4.3. Hematology

Blood analyses showed no specific effect of sonication (Table 3). The high plasma CPK, which was already demonstrated in the baseline measurement, is most likely caused by transportation of the animals just prior to the surgical procedure. The reduction of the CPK value to a normal value in 7 days after the transportation, confirms the assumption that the high CPK values are due to transportation. Free hemoglobin values did not reveal significant red blood cell destruction due to the sonication procedure and remained within normal levels in time.

4.4. Efficiency

The efficiency results show significant reduction of cerebral emboli due to EmBlocker™ activation. The ultrasonic wave of the EmBlocker™ exerts a more pronounced diversion effect on gaseous emboli than on the solid emboli, which can be explained by the difference in acoustic impedance (as mentioned before) between the two types of emboli and their surroundings.

However, both gaseous and solid cerebral emboli were reduced by use of the EmBlocker™, but despite the use of lower intensity of the EmBlocker™ for gaseous emboli, the reduction of emboli in the cerebral arteries is 10% higher for gaseous emboli than for solid emboli. The proof of principle was demonstrated by these preliminary results; yet adjustments of the positioning, the shape and size of the transducer...
could improve the efficiency. The EmBlocker™ seems to be an efficient device to reduce cerebral emboli during extracorporeal circulation and thereby could reduce postoperative neurological complications [4,5,12].

5. Clinical application

Neurological complications after cardiac surgery are well recognised and can vary from cognitive decline, with an occurrence of 5–43%, to stroke with an occurrence of 2–8% [1–5]. A correlation between cerebral emboloi and neurological complications has been described in the literature presuming that a reduction of cerebral emboli could reduce these neurological complications. Specific interventions during coronary bypass grafting (CABG) and open heart surgery were identified to generate most of the cerebral emboli; cannulation, cardioplegia needle incision, start and stop CPB, cross-clamping, cross-clamp release, side-clamping, side-clamp release, de-airing and de-cannulation [2]. According to the findings of this present study, the EmBlocker™ is able to divert solid as well as gaseous emboli to the descending aorta and reduce the number of emboli in the cerebral vessels. The EmBlocker™ should be placed after thoracotomy on the aorta ascendens above the bifurcation of the aorta and the innominate artery (Fig. 2). One minute of activation of the EmBlocker™ during the interventions mentioned before should be sufficient to divert the generated emboli. Because all interventions (except de-airing) are associated with solid emboli the higher intensity (1.5 W/cm²) of the EmBlocker™ is recommended. In open-heart surgery, there is an extra procedure in which cerebral gaseous emboli are expected. This time period is from the moment the heart starts ejection and can continue for approximately 5–10 min. Due to the fact that the emboli in this period are mainly gaseous emboli, the lower intensity level for 5 min is recommended. In this present study, the clinical activation time was examined twice as a result of which it can be concluded that the recommended clinical activation times could be used safely.

Although this animal study already demonstrates a diversion effect, a possible change in intensities or design of the EmBlocker™ could improve the efficiency.

6. Limitations

The high standard deviation of the number of emboli in the control injections, as shown in Table 4, could be due to the high standard deviation of the weight of the animals. The anatomy difference, particularly in innominate and in right common carotid artery, could cause a difference in absolute flow to the cerebral vessels. No flow measurements were done to confirm this hypothesis.

In this preliminary study a surrogate marker (number of emboli) was used to examine the efficiency of the EmBlocker™, no brain MRI or brain pathology has been performed to examine the cerebral damage of the emboli (gas and solid) injections.

7. Summary

Temperature, hematological and histopathological results show no effect although the clinically pretended period of activation of the EmBlocker™ was used twice. Efficiency measurements show a significant decrease in cerebral emboli during emboli injections during extracorporeal circulation. Use of the EmBlocker™ in cardiac surgery bears the potential to lower the risk of postoperative neurological complications.

Acknowledgments

The authors want to express their gratitude to Mr T. van der Nagel and Ms M. de Jong for their technical support and effort.

References

[10] Goldstein LS, Dewhirst MW, Repacholi M. Emboli and erythrocytes or any other particles, it’s not that much, it won’t be able to move that particles. That’s also why air is much better to divert because the acoustic impedance is quite large.

Appendix A. Conference discussion

Dr U. Lockowandt (Stockholm, Sweden): It’s quite fascinating to move objects with an ultrasonic beam.

Do you think it will influence the thrombocytes, lymphocytes or erythrocytes?

Dr Sauren: No. It was also tested. Because this mechanism only works when there is a difference in acoustic impedance and the difference between blood and erythrocytes or any other particles, it’s not that much, it won’t be able to move that particles. That’s also why air is much better to divert because the acoustic impedance is quite large.


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Dr P. Gründeman (Utrecht, The Netherlands): I think it’s a great idea to get the air down in the descending aorta. Suppose there is debris in the ascending aorta that’s attached to the inner wall, for instance, calcification, can that (material) be dislodged by this technology?

Dr Sauren: That is, of course, an interesting question, but it’s something we still need to take a look at. Of course, it’s different material then particles in the blood circulation, I mean, it’s attached to the aorta, if you’re talking about calcification. but it’s something we still need to take a look at for sure.

Dr Van Der Linden (Stockholm, Sweden): I have a question regarding the size of the particles you can redirect. How much power do you need to redirect big particles as opposed to microemboli, very small particles?

Dr Sauren: Well, this is something we are looking at in the in vitro setup actually. We want to use different kind of sizes. And this animal study, quite big particles, were used between 500 and 750 μ. And also we want to have a look at which power should be used for each different size. But I cannot say anything about it now.

Dr Van Der Linden: Did you use transcranial Doppler in these pigs insonating the middle cerebral artery or did you insonate the carotid artery?

Dr Sauren: No, I placed the probes on the eyes, because it was possible to get the cerebral arteries through the eyes.

Dr Van Der Linden: So you were looking at retinal emboli?

Dr Sauren: No, no. Because of the eye there is no bone at the spot where the nerves are going into the brain. There is a spot where there no bone is so I can get through with the ultrasound.

Dr Van Der Linden: Because the anatomy of the cerebral circulation of the pig is a bit difficult and it’s difficult to insonate the middle cerebral artery.

Dr Sauren: I’m aware that we are not completely sure that we have the middle cerebral artery and that is also why these results could be the results are not that perfect. Maybe the results could be better because of this, but we didn’t see them. So we still need to take a look at it further.

Dr Van Der Linden: And which Doppler technique did you use? Did you use the EmboDop?

Dr Sauren: Yes, it was the EmboDop.