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Alcor A-1661 Case Report Contents:

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1. Summary

Information is derived from multiple sources and is all converted to Mountain Standard Time (MST). For de-identification, dates are not shown. T-0 represents the date of pronouncement of legal death, T-X represents occurrences before T-0, and T+X represents occurrences following T-0.

A-1661 was a 57-year-old, female with neuro cryopreservation arrangements. She experienced cardiac arrest several days after minor surgery. The member was found unconscious and unresponsive in her home by a friend when she returned from having a prescription filled for the member. Paramedics and police were called to pronounce; the member was taken to a local emergency room at a hospital in Scottsdale, AZ where she was pronounced legally deceased.

Because the legal death was unattended, it was a medical examiner’s case, which can include an autopsy. Alcor worked with the Medical Examiner (ME) and the member was released to Alcor within hours after her legal death. She went into cryogenic cooldown on T-0 days, 10 hours and 19 minutes after her estimated time of cardiac arrest. Cooldown was terminated on T+24 days and the patient was transferred to long-term maintenance in liquid nitrogen on T+25 days.

2. Patient Assessment

January, T-0 days

Alcor received a call at 13:30 hrs from the home of an Alcor member who approximately one week earlier had a tonsillectomy and a turbinate cauterity to help her with breathing issues. Sitting in bed for a week after surgery was a risk factor for the member due to her history of PE, however she was not considered by Alcor to be at significant risk of death and was not receiving any type of standby from Alcor.

An Alcor staff member who was visiting in a personal capacity as a friend had gone out to fill a prescription for the member and when she returned at 13:05 hrs, she found that the member in an apparent state of cardiac arrest. The staff member called 911 to request paramedics and police; she was told to put the member on the floor and do CPR until the paramedics arrived. When the paramedics arrived at the house the member was in asystole. They transported her to the ER where she was then pronounced.

The member’s primary physician was on vacation and therefore could not promise the ME to later fill out the death certificate. Further, no Designated Power of Attorney for Healthcare (DPOA) was listed on the Alcor database.
3. Deployment

Alcor’s Medical Response Director (MRD), together with a skilled contract paramedic and another staff member who would serve as the scribe, left Alcor at 13:53 hrs with the response vehicle and all standby, stabilization and transport (SST) equipment. The MRD and contractor had been readying to leave for Laughlin, NV to give a training course but rescheduled that course and went instead to the hospital where the member had been taken.

From 13:55 hrs to approximately 17:00 hrs there was extensive discussion on Alcor’s internal communication system (ICS) regarding the following topics: lack of paperwork for the Health Insurance Portability and Accountability Act (HIPAA) due to the sudden and unexpected emergency, two Alcor staff members going to the member’s home to search for any possible paperwork located there, how to obtain contact information for the member’s physicians, the fact that the member’s primary physician was on vacation, attempts to contact the physician who would be taking over while the primary physician was gone, whether or not this would become a medical examiner’s case with a possible autopsy, providing eye witness information to the ME about how and when the member was found to help expedite the member’s release, trying for a virtual autopsy if needed and finally about getting a local attorney to call the ME to accelerate the member’s release.

At 13:59 hrs the member was pronounced legally deceased in the ER. In this case, the time of cardiac arrest was estimated to be at 12:50 hrs. The cause of death was unknown and no autopsy was performed.

When the Alcor team arrived at the ER at 14:11 hrs, the paramedics were manually ventilating the patient without chest compressions. The MRD asked for permission to apply the mechanical cardiac support device and place ice on the patient.

The Alcor team was informed at 14:18 hrs that they could not perform any procedures, even the application of ice to cool the member until the ME cleared it. The supervisor was asked why cardiopulmonary support (CPS) and ice could not be implemented if the paramedics were continuing to give her oxygen manually. The team was then given permission to apply CPS.

At 14:25 hrs the MRD put the CPS device on the patient but the unit would not start. The battery appeared to be the problem. It was plugged into the wall power supply but still would not work. In addition to the equipment problem, there had been a change to a less cooperative attitude on the part of the ER personnel.

An ER nurse informed the Alcor team at 14:34 hrs that their Risk Management office had been contacted and no procedures of any kind could be done until the ME released the patient, but an ER physician was attempting to call the member’s primary physician. The ER was very full with other emergencies and it was not likely that the staff would do more than passively wait for the ME’s office to call them. The ME’s office was also overloaded due to it being a holiday. This made a timely release less than likely and it seemed probable that the patient would not be released by the ME until at least the next day.
There had been extensive discussion on Alcor’s ICS about having a local Alcor attorney call the ME’s office to expedite the release of the patient. At 14:50 hrs, while still in the ER, the MRD called an attorney who had worked with Alcor in the past and asked that he be given a message that it was an Alcor emergency.

It was learned that the member’s recent surgery had been done at the same hospital where she was taken by ambulance for this emergency; therefore, the ER had access to the member’s surgical records. At 15:13 hrs the staff member who had found the member in cardiac arrest was asked to call the surgeon who had done the recent tonsillectomy and a turbinate cautery and give him her eyewitness information about the circumstances that led to the member’s cardiac arrest. That surgeon then called the ER physician to relay the information.

When the attorney returned the MRD’s call he was asked to contact a specific person in the ME’s office that Alcor’s CEO had spoken with several months earlier as a general goodwill call. She had said that if they understood a case was an Alcor case, they would cooperate. The attorney called the ME’s office but could only leave a message. He was told that Alcor’s case was in the hands of the Investigations Division and that since it was a holiday all the investigators were backed up.

At 15:55 hrs the ER announced an impending Code Blue, a medical emergency such as cardiac or respiratory arrest. The Alcor team was informed that the ER staff needed the room in which the team had been waiting with the Alcor patient. The patient would probably need to be moved into the hallway until released by the ME. The MRD asked for the patient to be moved to the morgue and placed in a cooler as soon as possible. The ER staff agreed. It was not optimal, but better than sitting in a hallway at room temperature without even ice to start cooling.

The patient was placed in a temperature-controlled room (at 2.67°C) at 16:22 hrs at the hospital morgue, where she would await release from the ME’s office. Because it was late in the day, it was possible that the release would not be obtained until at least the next day. The hospital morgue promised to call the MRD as soon as a release was obtained. The team then returned to Alcor. The Alcor operating room (OR) had been set up and was ready.

4. Transport

Alcor’s attorney called the ME’s office again at 17:26 hrs hoping that since they had a heavy holiday workload, someone might be working after hours. The strategy was successful, and this case was moved to the top of the ME’s case list. At 17:48 hrs the ME’s office called Alcor and spoke to a member of the Alcor staff who had been in touch with the member frequently over the past few days, according to the patient’s cell phone log. The ME was asked to call the staff member who had found the member in cardiac arrest, after which it was hoped that the ME would be able to make a determination on whether or not to release the member’s body to Alcor that night.
At 17:51 hrs Alcor’s MRD reported on the ICS that the ME had released the member and he was on his way to the hospital to retrieve the patient. An Alcor staff member was deployed to the hospital to assist the MRD. The Alcor OR team was asked to return to Alcor to prepare for cryoprotection. At 18:40 hrs the patient had been picked up and the team would soon depart for the 10-minute drive to Alcor.

5. Cryoprotectant Perfusion Surgery

The patient arrived at the Alcor operating room (OR) at 19:15 hrs. Ice bags had been placed around her head before she departed the hospital. The OR team was setting up tubing on the pumps. The new gurney that had been made for use in the Mercedes Sprinter response vehicle collapsed while going over a 1” lip on the floor of the doorway into the OR. The patient was not compromised.

In order to raise the patient’s head and better expose her neck for surgery, three 12” polyethylene supports were placed under the patient’s shoulders. The right and left sides of the patient’s face were marked for proper placement in the cephalic halo and for sampling line placement. A nasopharyngeal thermocouple probe was placed in the patient’s left nare at 19:23 hrs. The initial temperature after the probe was connected to the data acquisition system at 19:24 hrs was 28.4°C.

The decision was made by the OR staff at 19:26 hrs to bypass the administration of medications and ice bath cooling in order to prioritize getting the patient cooled more rapidly by flushing with cold B1 washout solution (see the Discussion section). The patient was draped for surgery and at 19:29 hrs the first surgical incision was made on the patient’s neck. The nasopharyngeal temperature (NPT) at 19:31 hrs was 29.1°C. No blood clots were seen in the effluent from the jugular veins which had been severed during the surgery.

The trachea was transected with surgical scissors and the left carotid artery was isolated, raised and tied off at 19:35 hrs. The right carotid artery was isolated, raised and tied off three minutes later. The cephalic isolation was initiated at 19:38 hrs and completed at 19:41 hrs. The weight of the cephalon was 5.28 kg. The cephalon was placed into the cephalic halo but not deeply enough; it would later require adjustment.

The data acquisition system was connected at 19:42 hrs; the NPT was 29.1°C. The system was not showing pressure data. The recycled pressure transducers had not been flushed and dried properly after the last case. The problem was corrected within a few minutes when another pair of pressure transducers was used to replace the faulty ones. The tubing circuit was switched from closed circulation to open circulation, drawing on the washout solution in the mixing reservoir. The main pump was placed on automatic control; the target pressure was set to 50 mmHg.
At 19:46 hrs the location of the left carotid artery was re-evaluated. The anatomical structures were not obvious. The left carotid artery could not be cannulated with a 14 French (Fr) red Robinson catheter, the size normally used, because the artery was unusually small. Several different sizes of cannula were tried before the cannulation was achieved with a 16 gauge angiocath and an adapter to attach the angiocath to the red Robinson catheter. The angiocath was secured with 3-0 silk. A 14 Fr red Robinson catheter was placed into the right carotid artery and secured at 20:13 hrs. A sampling line was set in the left jugular vein.

At 20:25 hrs; the mixing reservoir volume was down to 1 L. The main pump was switched to draw from the washout solution bladder.

At 20:28 hrs a blockage made placing the sampling line in the right jugular difficult; the cause of the blockage was not determined. The sampling line slipped from the right jugular vein and at 20:37 hrs the sampling line was again set in the right jugular vein and secured. Concurrently, the main pump was switched back to closed-circuit to reduce the reservoir volume again. At 20:43 hrs the NPT was 24.0°C.

The cephalon was repositioned to make the burr holes. The easily accessible area of the patient’s head was shaved where the burr holes were to be placed. Incisions were made in the scalp for the burr holes and at 20:49 hrs the left burr hole was drilled. The patient’s hair was twisted around the halo clamps. The patient’s hair was completely shaved while the cephalon was loose. The cephalon was repositioned and tightened in the halo. The halo ring fixture appeared to have been broken during setup. It was retightened and secured with red Loctite. At 20:58 hrs it was noted that neither of the sampling lines was drawing effluent.

6. Cryoprotectant Perfusion

Once circulation was established, the pump rate declined from 230 mL/min to less than 75 mL/min over the course of an hour. The pressure feedback loop controlling the pump was near the point of going into uncontrolled oscillation. The system pressure was 90 mmHg with near-zero pump speed. The pressure setting was increased to 100 mmHg, but the pump speed responded only slightly.

Alcor’s Chief Medical Advisor (CMA) was called at 20:58 hrs for advice on how to proceed with this lack of flow. It was proposed that to potentially prevent going to a straight freeze protocol (a cooldown to liquid nitrogen temperature without cryoprotectant perfusion), an attempt should be made to reduce the brain edema by perfusing the cephalon with a large bolus of cryoprotectant together with the two anti-coagulants, sodium citrate and heparin, and thereby improve flow. Two bolus injections were made 5.5 minutes apart. The first contained 150 mL of nM22 at 20 Brix concentration, 50,000 IU heparin, and 20 g sodium citrate. The second contained 100 mL of nM22 at 25 Brix concentration, 50,000 IU heparin, and 20 g sodium citrate.
Meanwhile, the right burr hole was drilled at 21:01 hrs using a Codman perforator; the perforator and the drill site were cooled with normal saline. The opening was cleared and expanded using a rongeur. At 21:03 hrs the main pump was switched to closed-circuit; 50,000 units of heparin and 20 grams of sodium citrate were added to the mixing reservoir to minimize coagulation. The mixing reservoir volume was 0.75 L and 150 mL of nM22 x 1.25 concentrate was added as a bolus. The cryoprotectant concentration rose rapidly to 20 Brix. The pump speed increased, but only slightly.

The right burr hole was completed at 21:09 hrs. 100 mL of nM22 cryoprotectant perfusate was added to the mixing reservoir at 21:10 hrs. This was a second bolus; the concentration of cryoprotectant rose rapidly to 25 Brix and the flow rate also increased, as the cryoprotectant step reached the patient, 600 mL and 7 minutes downstream from the arterial refractometer.

A thermocouple temperature probe was placed in the right burr hole and at 21:12 hrs the probe was connected to the data acquisition system; the NPT was 19.4 °C. The cryoprotective ramp was started at 21:18 hrs. The main pump speed was set to 16 (21 mL/min).

At 21:23 hrs the ramp pump was started on full speed to increase the mixing reservoir volume; the diverter had been in the wrong position and was removing volume from the mixing reservoir. The ramp pump speed was decreased to 50 (approximately 66 mL/min.). The sampling lines were still not taking samples due to there being very little venous return. The pump speed was increased to 60 (approximately 79 mL/min).

At 21:30 hrs 250,000 units of streptokinase were added to the closed-circuit perfusion. A single, manual refractive index reading was taken from the effluent at 21:32 hrs. The cryoprotectant concentration was 25.6 Brix. No flow could be detected from the left jugular vein. It was noted at 21:36 hrs that both corneas were partially collapsed.

Sidebar:
Per the cryoprotection protocol, the ramp is to be paused at 30 Brix (50% of the desired end concentration) to allow the patient to come to osmotic equilibrium. The neuroperfusion enclosure and the chiller are switched from +3°C to -3°C operation. At the end of the 30-minute pause the ramp is resumed at the maximum addition rate (maximum without losing total volume in the circuit) to go to 105% of the desired end concentration (52.5 Brix) and held between 102% and 105% concentration until, hopefully, the goal is obtained. Under the circumstances, none of this occurred.

It was noted at 21:45 hrs that due to the low flow rates experienced in this case, there would be no pause at 30 Brix (50% of the desired end concentration) to allow the patient to come to osmotic equilibrium and no pause for the sub-zero terminal concentration ramp. Cryoprotection was proceeding but was slow and not expected to be uniform.

A single, manual refractive index reading was taken from the effluent at 21:50 hrs. The cryoprotectant concentration was 35.9 Brix. The arterial concentration was 52 Brix and the right venous concentration was 15.58 Brix. The left venous concentration was not meaningful due to
the lack of flow. The main pump flow rate was 125 mL/min at 100 mmHg. The ramp pump was stopped.

At 21:55 hrs the patient’s eyeballs had started to collapse and there was considerable tanning around the nose, eyes and forehead. The location of the tanning could have been related to the patient’s recent surgery. Shrinkage of eyes and darkening of skin color are normal responses to cryoprotectant perfusion.

The ramp pump was switched back on at 22:06 hrs and at 22:19 hrs it was turned off again; the pump speed was nearly zero. A drop in the mixing reservoir level from 1.9 L to 1.0 L was started one minute later. At 22:26 hrs the ramp pump was turned back on at speed 60 (approximately 79 mL/min). At 22:35 hrs the ramp pump was stopped; the flow was so low that the main pump went into rapid oscillations. Edema, resulting in lack of perfusion, had returned.

Cryoprotection was terminated at 22:41 hrs. A single, manual refractive index reading was taken from the effluent at 22:36 hrs. The cryoprotectant concentration was 43.8 Brix. The lid to the cephalic enclosure was opened at 22:43 hrs; no brain volume reduction was observable through the burr holes.

Lines and tubing were removed from the cephalon in order to move it to the patient care bay for cooldown. A hole was drilled in the vertebra and an eyebolt was placed for ease of handling. The vertebra broke and this procedure needed to be redone.

At 23:00 hrs the final weight of the cephalon was 5.94 kg (5.94 kg -5.28 kg = 0.66 kg weight gain, or 12.5 percent).

7. Cooling to Liquid Nitrogen

The cephalon was lowered into the cooldown dewar. The thermocouple lines from the cephalon were taped to the side of the dewar and were extended in order to reach out of the dewar to be connected to the cooldown computer. The cooldown lid was placed on top of the dewar and taped to secure the closure. The thermocouple probes were connected to the data acquisition system and the circulating fan was activated.

At 23:09 hrs on T-0 days cryogenic cooldown was initiated using the program “Cryoprotected Neuro”, which plunges to -110°C and then proceeds to LN₂ at -1°C/hour. Isotherms were noted in both the burr hole and nasopharyngeal temperature plots, between -3°C and -9°C, indicating substantial ice formation. There was also an unusual stretch of retarded cooling, down to about -23°C, probably indicating ice formation at some distance from the thermocouple junction locations.
On T+6 days at 01:45 hrs cryogenic cooldown was terminated at liquid nitrogen temperature. A CT scan was made of the patient’s brain on T+24 days under liquid nitrogen. The patient was transferred to a long-term care dewar on T+25 days.

8. Timeline and Time Summaries

Timeline

January, T-0 days

12:50  Estimated time of the cardiac arrest
13:59  Pronouncement of legal death
17:51  Patient released by Medical Examiner
19:15  Patient arrived at Alcor OR
19:24  Initial nasopharyngeal temperature (NPT) after data acquisition connected: 28.4°C
19:29  Start of surgery for cephalic isolation
19:41  End of surgery and weight of cephalon = 5.280 kg
19:46  Left carotid cannulation problems
20:25  Start of open-circuit washout
20:28  Difficulty placing sampling line into the right jugular vein
20:58  Sampling lines not drawing perfusate
21:03  More aggressive increase in CPA osmolality to mitigate ischemia-induced perfusion compromise
21:09  Right burr hole completed
21:18  Cryoprotective ramp pump started
22:41  Termination of cryoprotection; 43.8 Brix
23:00  Weight of cephalon was 5.94 kg (-5.28 kg = 0.66 kg gain)
23:09  Start of cryogenic cooldown

January, T+6 days - Termination of cryogenic cooldown at LN₂ temperature

January, T+24 days - CT scan of the brain at LN₂ temperature

January, T+25 days - Transferred to long-term maintenance at LN₂ temperature
Time Summaries

Stabilization and Transport

hrs: mins
06:25  From estimated cardiac arrest to patient arrival at Alcor: 12:50 hrs to 19:15 hrs
05:50  From estimated cardiac arrest to Medical Examiner’s release: 12:50 hrs to 18:40 hrs
03:34  From estimated cardiac arrest to entering cooler at 36.8°F: 12:50 hrs to 16:24 hrs
02:16  Total time in the morgue cooler: 16:24 hrs to 18:40 hrs

Cryoprotective Surgery

hrs: mins
00:14  From arrival at Alcor to the start of surgery: 19:15 hrs to 19:29 hrs
00:12  From the start of surgery to the end of the cephalic isolation: 19:29 hrs to 19:41 hrs
01:49  From the start of surgery to the start of the cryoprotection: 19:29 hrs to 21:18 hrs
03:12  From the start of surgery to the end of the cryoprotection: 19:29 hrs to 22:41 hrs

Cryoprotective Perfusion

hrs:mins:
01:38  From the start to the end of cryoprotection: 21:03 hrs to 22:41 hrs
00:28  From the end of cryoprotective ramp to the start of cooldown: 22:41 hrs to 23:09 hrs
03:54  From arrival at Alcor to the start of cooldown: 19:15 hrs to 23:09 hrs
08:28  From the estimated time of cardiac arrest to start of cryoprotection: 12:50 hrs to 21:18 hrs
10:19  From the estimated time of cardiac arrest to start of cooldown: 12:50 hrs to 23:09 hrs

9. Discussion

No Health Insurance Portability and Accountability Act (HIPAA) or Power of Attorney (POA) forms were available, making it difficult to obtain medical information from the emergency room (ER). There needs to be more effort to inform the membership about the need to have them fill out POA and HIPAA forms and send them to Alcor for their files. There is a Durable Power of Attorney (DPOA) form on the Alcor website and members need to be informed of the importance of gaining HIPAA forms from their local hospitals, filling those out (without dating them) and sending them to Alcor for their files. An article about this needs to be published in Cryonics magazine (this would be a second request in the magazine for membership involvement in this
issue for their own benefit) and the group discussed obtaining HIPPA forms for the local Scottsdale hospitals so that employees can have these available.

The Circle of Willis is an anatomical vascular structure within the human brain that allows collateral circulation through alternate vessels in the brain if the flow is reduced due to injury to the major vessels. Approximately 1% to 17% of humans have an incomplete Circle of Willis. There are two paired arteries that supply blood to the brain, the carotid arteries, and the vertebral arteries. When cannulating a neuro cryopreservation patient, if the Circle of Willis is not complete, the vertebral arteries need to be cannulated as well as the carotid arteries in order to get complete circulation in the brain.

For decades, an incomplete Circle of Willis was not seen in the Alcor operating room (OR). The perfusion circuit had the capability of perfusing both the carotid and the vertebral arteries but it had never been used. On this case, there was no note made in the OR scribe notes that there was a check to see if the Circle of Willis was complete, therefore we have to assume that the check was not made, perhaps due to an incomplete Circle of Willis being rare, and also in part due the other anatomical problems that were complicating this cannulation. Nonetheless, an assumption was made that it was intact and the vertebral arteries were clamped to keep the Circle of Willis pressurized in order to enhance circulation. Pursuant to this case, Alcor now has an improved capability to cannulate the vertebral arteries should this be required.

Due to the low perfusion flow rates experienced in this case, there was no pause at 30 Brix (50% of the desired end concentration) to allow the patient to come to osmotic equilibrium and no pause for the sub-zero terminal concentration ramp. Cryoprotection was proceeding but was slow and not expected to be uniform. Lowering the perfusate and cephalic enclosure temperatures to subzero would probably have induced ice formation in areas of the brain with poor cryoprotection. It had become obvious that the blood-brain barrier (BBB) was compromised, without which staying ahead of the edema would only be temporary.

Very little venous return was achieved during cryoprotectant perfusion. In order to reduce edema in the brain and possibly attain better perfusion, the assistant perfusionist recommended that a bolus of cryoprotectant be given; the Chief Medical Advisor (CMA) agreed to this effort. The flow rate more than doubled, but gradually declined again over an hour and a half. Perfusion was finally halted when the pump control circuit went into an oscillation.

Since it is not likely to be the only time this situation will occur, some discussion about this alternative perfusion plan is needed for future use. From the plot “A-1661 cryoprotection, [nM22] and pump rate”, most of the counter to the edema occurred in the initial steps to 25 Brix. Whether or not the later steps had any effect is less certain. Most cases that end because of low flow occur in the terminal portion of the cryoprotection, but early edema is occasionally observed, and here large cryoprotectant steps have been shown to be effective in temporarily and usefully overcoming edema.

It is likely that 2-4 hrs of normothermic ischemia caused the blood-brain barrier (BBB) to break down. Compromise of the BBB leading to cerebral edema during cryoprotectant perfusion is a known result of long periods of ischemia and this has been seen in many Alcor cases.
There was a volume of approximately 600 mL, of filters, HEX, and tubing between the arterial refractometer and the patient, resulting, particularly at low flow rates, in a considerable amount of time between action and effect. This problem will be corrected in the new compact neuro perfusion system under development, but in the meantime, the refractometer will be moved nearer to the patient. This move is also important in correlating cryoprotectant concentration and brain retraction measurements.

There was a long, several hours, delay in beginning to cool down the patient. This likely led to unfavorable degradation of her tissues. For example, consistency of the dura typically seen in these patients is a firm, pliable membrane, similar as one would see with intact wax paper. In this case, her dura had already partially decomposed. It appeared as small, friable portions of tissue. It was not possible to piecemeal dissect away all of the fronds of tissue under the burr hole. With no clear path to the visualization of the brain, the laser monitor could not be used to follow the shrinkage of her brain.

An unidentified white, curdled-like substance was oozing from the burr holes. This peculiar matter was seen when the burr holes were first made, even before perfusion started. Throughout the entire procedure, the surface of the brain was never possible to visualize. This is something that has rarely, if ever, been seen before at Alcor. The interpretation is unclear because debriefing discussion participants were not aware of any pathology literature documenting such drastic change in the appearance of cerebral grey matter (if that’s what it was) over such a short post-mortem time period.

At the termination of the cryoprotective ramp, the final weight of the cephalon was 5.94 kg (5.94 kg -5.28 kg = 0.66 kg weight gain, or 11.1 percent gain). This weight gain is consistent with BBB breakdown, brain edema and lack of flow that were experienced throughout this case.
10. Graphs and CT scans

![Graph of A-1661 cryoprotection](image)
Cryoprotectant Distribution
The CT scans were made on T+24 days while the patient was in liquid nitrogen (-196°C).

The CT scans showed no reduction in brain volume. Cryoprotectant distribution was heterogenous with higher concentrations near the cortex of the brain. The core of the brain shows concentrations of CPA below what is necessary for vitrification. The cooling curve for the plunge to -110°C also indicates the formation of ice.