Alcor A-1261
Case Report

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Alcor A-1261 Case Report Contents:

1. Summary

Information was derived from multiple sources and was 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-1261 was a 69-year-old male with neuro cryopreservation arrangements. He had suffered from pancreatic cancer for about 6 months and experienced an aortic rupture. The death certificate only stated that the cause of death was due to natural causes.

The patient was pronounced clinically deceased in New York at 20:25 hrs on T-0 days in July of 2000. His estimated time of cardiac arrest was 18:30 hrs. He was flown to Alcor on T+1 days for cryoprotection. Cryogenic cooldown was initiated at 07:00 hrs on T+2 and terminated at 10:12 hrs on T+9 days. The patient was transferred to long-term maintenance at liquid nitrogen temperature at 16:15 hrs on T+20 days.

2. Patient Assessment and Pre-Deployment

This report was written in 2021 but the case took place in 2000; some details are no longer available. There were several scribes, their watches were not synchronized. An effort was made to use the time for events that provided the most consistent flow of events.

The member had pancreatic cancer for 1.5 years but had deteriorated in the last 30 days after having had an aortic rupture. Cancer had metastasized to his esophagus. He was receiving heparin daily by I.V. His medical surrogate, and close friend, worked with Alcor to set up standby arrangements.

T-0 days

The member experienced cardiac arrest at home before standby arrangements had been completed. On the direction of the physician who had said she would pronounce legal death, the medical surrogate called 911. The member was taken to the emergency room (ER) and was pronounced legally deceased at 20:25 hrs. Three physicians affiliated with Alcor made phone calls to the medical surrogate, the member's primary care physician, the emergency room (ER) physicians and the Medical Examiner (ME) and as a result, the autopsy was waived but no one could be persuaded to apply ice to the patient or to administer heparin or streptokinase while he was in the hospital morgue. The field notes do not refer to this being done by the funeral director.

3. Transport

A funeral director who had worked with Alcor for an earlier patient in New York agreed to expedite the transport of the patient to Alcor as soon as possible. However, two things resulted in the patient not being transported immediately to Alcor: 1) as it was a holiday weekend, the available commercial air flights were not optimal, and 2) the patient had changed his name and this resulted in the death certificate having one name while the hospital records used a different name. This confused the hospital morgue and resulted in delayed identification of the patient before he could be released to the funeral director. This further resulted in earlier airline flights being missed. 27 hours and 12 minutes elapsed between the patient's estimated time of cardiac arrest and his arrival at Alcor.

No one could be persuaded to apply ice to the patient or to administer heparin or streptokinase while he was in the hospital morgue. The field notes do not refer to this being done by the funeral director either. Anticoagulants administered to the member before cardiac arrest appear to have been sufficient to prevent most clotting.

4. Cryoprotectant Perfusion Surgery

Two timelines were made by different scribes and the times therein were not always consistent. The differences were not off by more than a minute or two.

T+1 days

The patient arrived at Alcor at 21:42 hrs. The operating room (OR) team was still setting up new equipment that had been brought from a research laboratory in another state and was checking out the perfusion circuit, refractometers and other equipment. The patient was placed in the surgical table containment tray in a light body bag. Bilateral burr holes were made in the patient's skull at 23:30 hrs for the placement of temperature and crackphone probes. It was noted at 23:37 hrs that blood was coming from both burr holes. The surgeon observed it was the veins bleeding out.

In order to initiate cryoprotectant perfusion as soon as possible, the patient's carotid arteries were cannulated before the cephalic isolation by using a six-foot extension to the tubing. The first incision for the cannulation of the right carotid artery was made at 23:38 hrs, and the carotid was raised at 23:49 but could not be cannulated yet because the initially selected cannula was too large. At 23:50 hrs the data acquisition system was online and the first nasopharyngeal temperature (NPT) reading of 14.4°C was recorded. The high arrival temperature suggests incomplete packing in ice during transport, about which no record is available. The first incision for the left carotid artery was made at 23:52 hrs.

T+2 days

For this case, a new organ research perfusion circuit was put together from an existing sterilized perfusion circuit, and 16.8 liters of 4% v/v glycerol-MHP-2 and 30 liters of 75% v/v glycerol-MHP-2 were batched up and filter-sterilized. Filtration of the 4% CPA began very shortly before the beginning of washout and continued throughout the washout, with the filtration of the 75% CPA ending slightly after the beginning of the CPA ramp.

A slow drip flow of perfusate was called for at 00:08 to prime the line ahead of cannulation. The left carotid was raised at 00:09 hrs and cannulated at 00:28 hrs. The left jugular vein was severed at 00:32 hrs. Just before the onset of perfusion, at 00:33 hrs, the arterial temperature was 12.8°C. Washout began at 00:36 hrs at an initial pressure of 20 mmHg and a temperature of 11.8°C. According to the first graph below, washout began with B1 containing 4% v/v glycerol (nearly 0.5M glycerol). At 00:45 the arterial temperature (AT) was 10.6°C and the venous temperature (VT) was 11.7°C.

No blood clots were observed coming out of the burr holes. There was a large amount of clear fluid coming out of the right burr hole. The right carotid artery was successfully cannulated at 01:02 hrs and perfusion of the right carotid was initiated at 01:03 hrs. The cannula was secured by ligating the artery onto it. Cutting of the right jugular vein was delayed until 01:16 hrs due to concern that the resulting outflow would obscure the surgical field before the cannulas could be secured.

The vessels were ligated onto both cannulae twice to secure them inside the vessels. Cutting the right jugular then resulted in a reasonable fluid outflow from both cut ends of the jugular and an increase in arterial flow rate was noted. Effluent was allowed to flow freely when perfusion was initiated. Selective perfusion through the left carotid line resulted in right jugular outflow, implying patency of the Circle of Willis.

At 01:22 hrs the cephalon was positioned so that the vertebral arteries were facing upward to prevent air from entering the vessels while they were being cannulated. This was the first neuropreservation case for which the vertebral arteries were cannulated. It was also the first time an isolated cephalon was perfused. The cannulation manifold was taped to the tub in such a way that it hung over the cephalon.

The cephalic isolation was initiated just below the cannulation site at 01:27 hrs. The perfusion manifold was held in the air because the patient's neck was too short and made it difficult to make incisions without disturbing the cannulae. The cephalic isolation was completed at 01:30 hrs. The cephalon was not weighed as it was not standard practice at the time. The cannulated cephalon was moved to the neuro perfusion container and mounted in the cephalic ring with the severed vessels up. Perfusion continued during this transfer.

It was noted at 01:36 hrs that the cannulae in the carotid arteries had moved and caused a blockage resulting in a loss of arterial pressure. The cannulae were adjusted and the arterial pressure temporarily shot up to 60 mmHg and then settled at 32 mmHg. The left vertebral artery was identified and isolated at 01:40 hrs. The perfusion pump was turned off at 1:43 hrs to

facilitate isolation of the right vertebral artery, which was exposed at 01:44 hrs and cannulated at 01:50 hrs.

The venous temperature probe in the jugular vein slipped loose. It was placed back into the jugular vein at 01:56 hrs. The refractometer line was drained for better readings. The left vertebral artery was cannulated and secured onto the cannula at 02:00 hrs. Left vertebral perfusion began at 02:03 hrs at a pressure too low to read, and at 02:04 hrs, all flow was diverted from both carotids to the left vertebral to prevent bubble introduction. At 02:05 hrs, the arterial temperature was 15.3°C, so the temperature of the heat exchanger was decreased to further lower perfusate temperature. Perfusion stopped at 02:11 hrs and resumed at 02:12 hrs, with an arterial temperature of 9.9°C (see the Discussion section for more details).

5. Cryoprotectant Perfusion

All perfusion up to this point had been open-circuit for washout. The circuit was closed and recirculation began at 02:13 hrs. Prior to starting the cryoprotective ramp, recirculating perfusion was used to lower the perfusate temperature to 10°C. The cryoprotectant ramp was initiated at 02:28 hrs, with the cephalon in a horizontal position and an AT reading of 9.3°C two minutes before. The arterial pressure was 32 mmHg at 02:30 hrs and 60 mmHg at 02:32 hrs. A jugular probe slipped out of the vein and was replaced at 02:35 hrs.

Glycerol addition was paused at 02:38 because it was found to have been proceeding more rapidly than intended, perhaps due to the relatively low mixing reservoir volume. Perfusion without further glycerol addition continued at a pressure of 48-52 mmHg. The filters were bypassed and changed by 02:44 hrs. The brain was noted to have receded away from the skull by approximately 1-2 cm at 02:43 hrs. Glycerol addition was resumed at 02:44 hrs after first lowering perfusion pressure to zero to avoid any pressure surges. By 02:45 hrs, the pressure was back up to 35mmHg, with some fluctuations, and by 02:46 hrs, pressure fluctuated between 40 and 60 mmHg. Pressure continued to be adjusted, and by 02:59 hrs pressure was 43 mmHg, AT was 9.7°C.

The machine-read venous concentrations were false since they began at approximately -3M, which was physically impossible. This resulted in not being able to make a meaningful graph of the venous refractive index. At 03:13 hrs, venous effluent samples were taken for freezing point determinations to resolve problems with venous refractometry. At 03:21 hrs, esophageal temperature tracking was discontinued at DuaLogR reading number 245 due to observation of no change in apparent temperature for an extended time.

A thermocouple temperature probe was placed in the left burr hole at 03:34 hrs. It was noted that the brain was now expanding and touching the inside of the burr hole. In response, the heat exchanger temperature was lowered to seek a target of -3°C to -5°C. At 03:43, pressure had risen to 70 mmHg, prompting a reduction of flow to correct this.

At 04:08 hrs the cryoprotectant ramp was paused to allow the brain tissue to equilibrate, and at 04:36 hrs the ramp was resumed at higher speed. It was noted that the brain was swollen and vascular resistance continued to increase (no numerical details were recorded).

Cryoprotectant perfusion was terminated at 06:19 hrs due to low flow rates produced by the high viscosity of the glycerol perfusate (given the need to limit maximum pressure), which made it impossible to keep cephalic temperatures below 10°C. The final cryoprotectant concentrations were, based on machine refractometry: arterial, 7.05 molar (M), venous, 4.07 M. However, later determinations based on refractometry of saved perfusate samples and calibration between 4 and 75% v/v glycerol showed a final arterial concentration of 7.75M (50.6 Brix, 56.6% v/v) and a final venous concentration of 7.42M (48.8 Brix, 54.3% v/v). The final temperatures were: arterial, 12.6°C, and venous, 14.5°C.

6. Cooling to Liquid Nitrogen Temperature

This case took place before a computerized cooldown system was created in 2006 and utilized a silicone oil bath and dry ice. The cryogenic cooldown was initiated at 07:00 hrs on T+2 days and was terminated at 10:12 on T+9 days. The patient was transferred to long-term maintenance at 16:15 hrs on T+20 days.

On the cryobiologist's advice, the cryogenic cooldown was initiated at -35°C, with an extended initial equilibration period to minimize the risk of intracellular ice formation at lower temperatures and achieve more uniform ice distribution than would be achieved if Alcor had immediately commenced cooldown (its former procedure). The subsequent cooldown to -50°C (see the explanation accompanying the temperature plot) took place at an average rate of roughly -21°C/hr. For reference, 7.1M glycerol, which is less than the venous concentration for this patient, can vitrify at a cooling rate of approximately 10°C/min.

The pharyngeal cooldown curve shows no visual evidence of ice crystallization. The final glycerol concentrations, which are equivalent to glycerol mole fractions of 0.29 and 0.32 for venous and arterial concentrations, respectively, both have freezing points below -47°C [1]. The patient temperature profile during cooling was not inconsistent with vitrification.

Crackphone readings were not obtained for this case.

7. Timeline and Time Summaries

Timeline

T-0 days

18:30 (est) Estimated time of cardiac arrest (when 911 was called after member found)
20:25 Pronouncement of legal death by paramedics

T+1 days

21:42 Arrival of the patient at Alcor
23:00 (est) Start of the burr hole surgery
23:30 Completion of burr hole surgery
23:38 Start of the surgery for cannulation
23:50 NPT probes attached to the data acquisition system (initial NPT 14.4°C)

T+2 days

00:36 Start of the open-circuit washout
01:22 Start cephalic isolation
01:30 Completion of cephalic isolation (cephalon not weighed)
02:00 Completion of surgery for cannulation
02:13 (est) Completion of open-circuit washout
02:28 Start of the cryoprotectant perfusion
04:08 Cryoprotectant ramp stopped for 30 minutes of equilibration
04:36 Cryoprotectant ramp restarted
06:19 Termination of cryoprotection (a final arterial concentration of 7.75M (50.6 Brix, 56.6% v/v) and a final venous concentration of 7.42M (48.8 Brix, 54.3% v/v).
07:00 Start of the cryogenic cooldown

T+9 days

10:12 Completion of cryogenic cooldown at LN_2 temperature

T+20 days

16:15 Transfer of patient to long-term maintenance at LN_2 temperature

Time Summaries

Surgery and Washout

hrs: mins

01:55 From the estimated time of cardiac arrest (ETCA) to pronouncement of legal death: 18:30 hrs to 20:25 hrs

27:12 From the estimated time of cardiac arrest (ETCA) to the patient's arrival at Alcor: 18:30 hrs on T-0 to 21:42 hrs on T+1

28:30 From ETCA to start of surgery: 18:30 hrs on T-0 to 23:00 hrs on T+1

03:00 From the start of surgery to the end of surgery: 23:00 hrs on T+1 to 02:00 hrs on T+2

30:06 From ETCA to start of washout: 18:30 hrs on T-0 to 00:36 hrs on T+2

01:45 From the start of washout to end of washout: 00:36 hrs to 02:21 hrs

31:43 From ETCA to end of washout: 18:30 hrs on T-0 to 02:13 hrs on T+2

Cryoprotectant Surgery and Perfusion

hrs: mins

01:18 From arrival at Alcor to the start of surgery: 21:42 hrs to 23:00 hrs

02:30 From the start of surgery to end of the cephalic isolation: 23:00 hrs on T+1 to 01:30 hrs on T+2

00:08 From the start to the end of the cephalic isolation: 01:22 hrs to 01:30 hrs

03:28 From the start of surgery to the start of the cryoprotection: 23:00 hrs on T+1 to 02:28 hrs on T+2

07:19 From the start of surgery to the end of the cryoprotection: 23:00 hrs on T+1 to 06:19 hrs on T+2

31:58 From ETCA to start of cryoprotection: 18:30 hrs on T-0 to 02:28 hrs on T+2

04:46 From arrival at Alcor to the start of cryoprotection: 21:42 hrs on T+1 to 02:28 hrs on T+2

03:51 From start to the end of cryoprotection: 02:28 to 06:19 hrs

00:41 From the end of cryoprotection to the start of cooldown: 06:19 hrs to 07:00 hrs

36:30 From ETCA to start of cooldown: 18:30 hrs on T-0 to 07:00 hrs on T+2

09:18 From arrival at Alcor to the start of cooldown: 21:42 hrs on T+1 to 07:00 hrs on T+2

8. Discussion

With more than 27 hours between cardiac arrest and arrival of the patient at Alcor, the probability of successful cryoprotectant perfusion with then-current procedures and cryoprotectant solutions was considered to be very low and a cryopreservation with no cryoprotection procedure (straight freeze), which would stop further deterioration, was initially considered most likely to offer the best outcome for this patient.

However, a cryobiology laboratory was studying a new surgical approach and a new non-glycerol cryoprotectant formulation that might result in vitrification (turning the body water into a glass-like solid rather than crystallization) of a cephalon. Alcor had already begun the process of upgrading its capability with these new advances and expected to have this capability available within the next month. But with assistance offered by the cryobiology researchers on their new surgical procedure, the use of a more ambitious glycerol cryoprotection protocol (see also “Getting to 8M glycerol and other perfusion problems” by Hugh Hixon in Cryonics Magazine, November 1993) and the use of a new perfusion manifold loaned to Alcor for this case, the decision was made to attempt to cryoprotect with glycerol, and if possible, even to vitrify this patient despite the high viscosity of glycerol.

During the cannulation procedure, at 02:04 hrs, the consulting cryobiologists had all the vessels clamped off except the left vertebral artery to try to prevent air bubbles from forming. That might have been (the notes are not definitive) because the arterial temperature was 15.3°C and they planned to run the heater-cooler to a lower temperature. When cold perfusate is allowed to warm up inside a perfusion line, it often outgasses, forming bubbles. Due to the warming that was noted, that might have been seen as a danger in the left vertebral line, a danger that would have been prevented by increasing the flow rate in that line so that the line would stay cold and prevent bubbles from forming or dislodge bubbles that had formed. Running the bypass line would also help to cool the vertebral line since the bypass line brings cold perfusate to the manifold that feeds the vertebral line, and cooler perfusion temperatures were desired. Since the bypass line that delivered cold solution to the manifold was still too warm but was being cooled, it is possible the pump feeding the manifold was turned off for 1 minute to allow time for the fluid in the bypass line to cool so that the fluid available for the vertebral line would be colder. At 02:05 hrs, the temperature reading was 15.3°C and at 02:12 hrs, at the same moment the main pump was turned back on again, the notes state that the temperature was 9.9°C, which was a significant improvement. Thus, waiting for that 1 minute between 02:11 hrs and 02:12 hrs might have been for the purpose of preventing the brain from being perfused with warmer perfusate.

It was observed at 02:24 hrs that for neuro cases it would be better to put the burr holes in the back of the head, just behind the ears, so the brain could more easily be observed without the need to turn the cephalon each time (the location of the burr holes for this case was in the superior temporal region bilaterally).

Just after the cryoprotectant ramp was initiated, it was discovered that the ramp was proceeding more rapidly than intended. This was due to the presence of only 2 liters of base perfusate (4% v/v glycerol) in the mixing reservoir upon closing the perfusion circuit following perfusion of 14

liters open circuit before that point. The normal perfusion procedure was geared for whole body perfusions involving a much larger mixing reservoir volume than 2 liters and was not corrected for this relatively low residual mixing reservoir volume.

During cryoprotectant perfusion, venous effluent samples were taken for freezing point determinations to resolve problems with venous refractometry, but it was not feasible to obtain melting points on these samples in real-time due to the lack of a person to monitor the thermal histories of the samples after placement in a freezer (and Alcor's osmometer was not working).

Before this case, Alcor had used a surgical technique developed by Alcor's then primary surgeon, whose experience was in cardiac bypass surgery. That was an open-heart surgical approach (median sternotomy with arterial cannulation of the aortic arch and venous cannulation of the right auricle through the right auricular appendage), except that the arms and lower body were blocked from perfusion (and cryoprotectant costs reduced) by tourniquets on the upper arms and clamping the descending aorta at the aortic arch. The cephalic isolation was done post cryoprotectant perfusion.

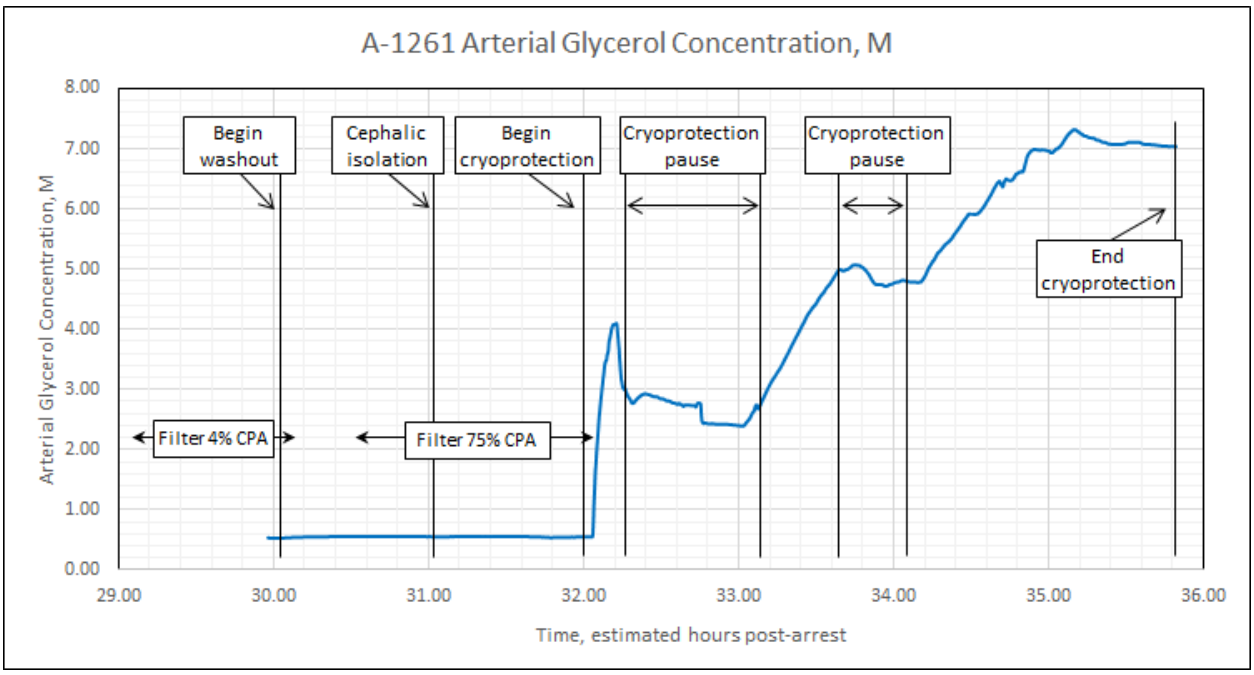
It had been proposed previously that it would be faster and less complex to cannulate the carotid arteries and perhaps the jugular veins. Alcor's first primary surgeon, and later, the presiding surgeon at the time of the present case, did not agree because 1) in the event the Circle of Willis was not patent, carotid perfusion alone could be insufficient without including the ability to separately perfuse the rear of the brain through the vertebral arteries, and 2) if the carotids were surgically damaged, or were not patent, there would be no alternative way to perfuse the brain, whereas starting at the aortic arch would enable later carotid cannulation as a backup approach if problems were encountered and this was needed. By necessity and logic, these arguments were accepted.

For this case, however, it was proposed that neuro cryoprotection be done on the isolated cephalon, using the following sequence of steps: cannulation of the carotid arteries, initiation of washout and cooling, cephalic isolation, cannulation of the vertebral arteries, and finally, cryoprotectant perfusion. Direct cannulation of the carotid arteries was expected to be advantageous particularly given the long postmortem delay before stabilization and the fact that any blood clots between the aorta and carotid arteries would not be pushed by perfusion into the carotid arteries and brain. Venous drainage through freely draining jugular veins would result in lower venous pressure inside the brain, increasing the perfusate flow rate, decreasing cerebral edema, and it was later discovered, decreasing leakage from bridging veins on the cortical surface, compared to venous drainage by thoracic cannulation of the right atrium of the heart.

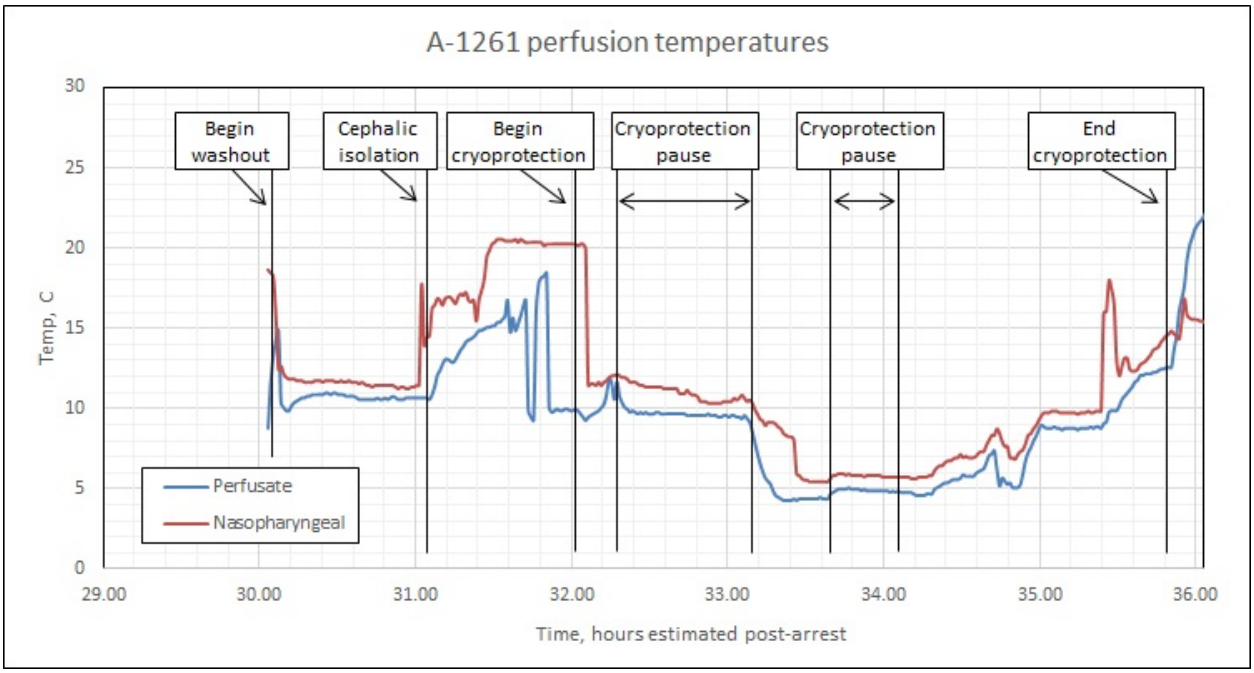
These new surgical techniques were intended to be carried out in connection with new non-glycerol-based cryoprotectant solutions to provide for vitrification of the human brain, which had never been attempted before. Although these new solutions and new equipment for rapid vitrification cooling weren't yet available at the time of this case, it was decided to at least implement the new surgical and perfusion procedure using Alcor's standard glycerol-based cryoprotectant solutions and determine how close the team could come, without the new solutions, to the goal of a glycerol concentration high enough to prevent ice from forming on cooling (vitrification).

Thus, this case was the first deliberate attempt to determine if vitrification was an achievable goal, as indicated by what could be accomplished even given the poor circumstances of this case and the makeshift nature of the technology applied. The results showed that, despite falling short of a formally vitrifiable concentration, the prospects for achieving such concentrations in future cases were excellent, and this optimism was borne out by a follow-up case 5 months later in which this goal was in fact achieved (the case of A-1502).

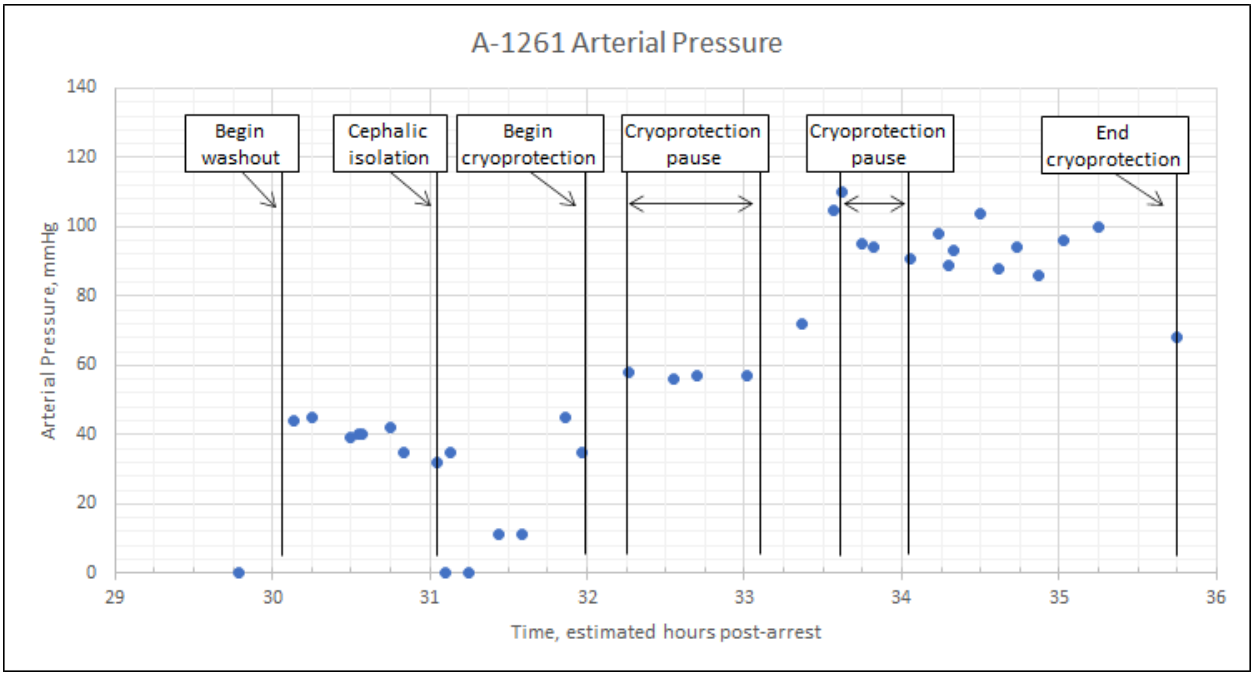
9. Graphs

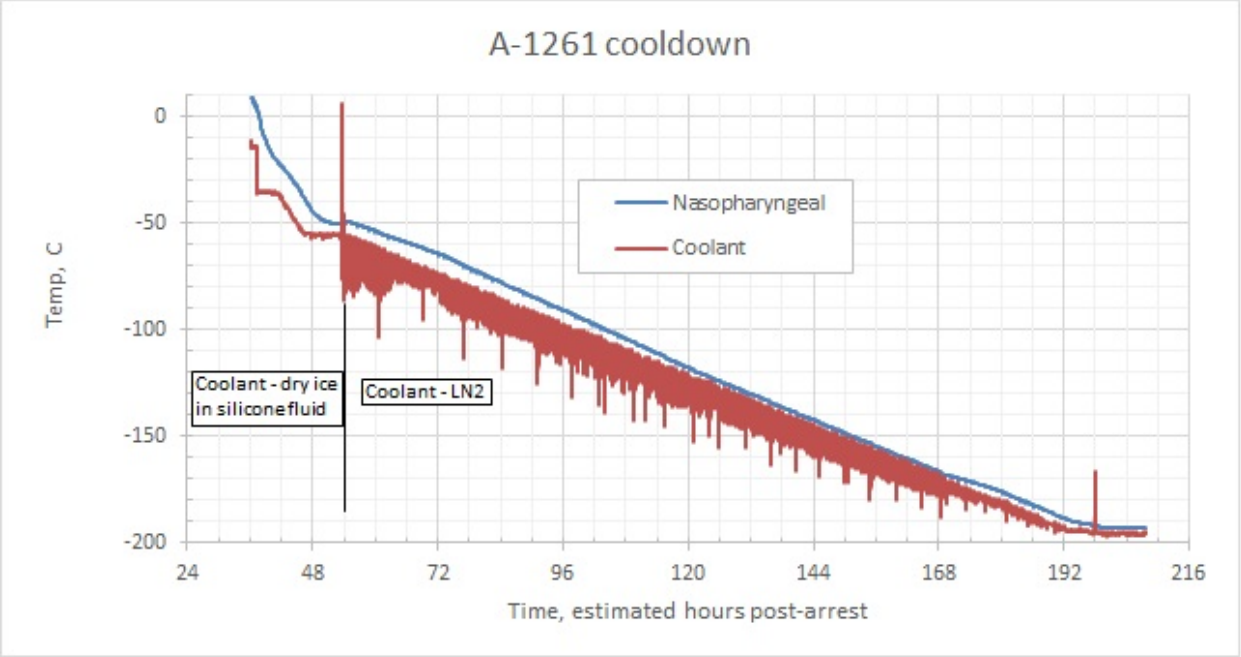


Note: The machine-read venous concentrations were false since they began at approximately -3M, which was physically impossible. This resulted in not being able to make a meaningful graph of the venous refractive index.

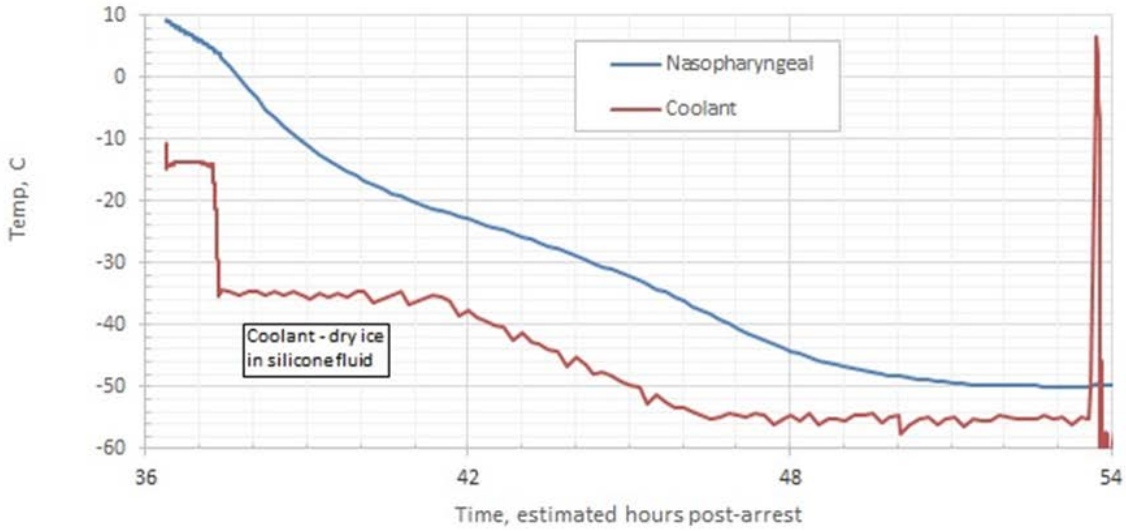


Note: The nasopharyngeal probe came out between 31.4 and 32.1 hours, and again between 35 and 36 hours, recording air temperature during those times.



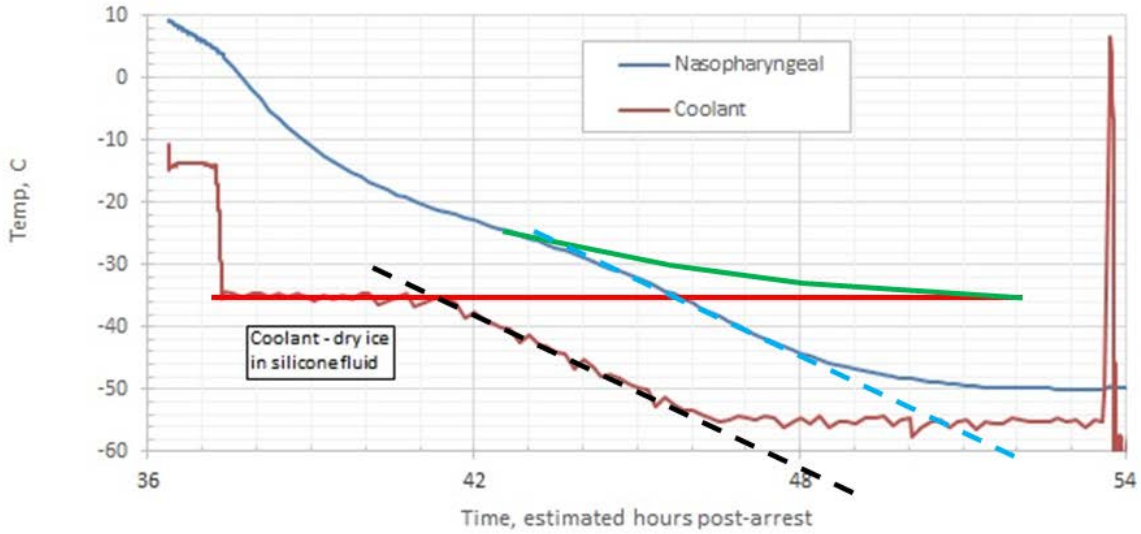


A-1261 Cooldown – plunge to -50°C



Note: Detail of the early part of the cooling process, showing the cooling response of the cephalon (nasopharyngeal temperature) to a two-stage cooling protocol (holding at -35°C for about 4 hours followed by linear cooling to -55°C.) The cephalon temperature descent shows a slowing between -5°C and -30°C that could be interpreted as being due to ice formation.

A-1261 Cooldown – plunge to -50°C



Note: Interpretation of the initial cephalic cooling curve in terms of the driving forces for cephalic cooling rather than as an indication of ice formation. Down to -25°C, the temperature can be seen to follow an exponential decline toward -35°C as illustrated by the green extension of the cooling curve to the first holding temperature (red line), showing the expected course had the temperature not been lowered below -35°C. There is no sign of any slowing of cooling in response to ice formation. Starting near hour 41, the cooling bath temperature was lowered at a constant rate (indicated by the dashed black line) and, about two hours later, the effect of this change in environmental temperature begins to affect the cooling rate deep in the cephalon. By hour 45, the cooling rate within the pharynx becomes equal to the cooling rate of the environment (the slope of the dashed blue line equals the slope of the dashed black line), demonstrating that the acceleration of cephalic cooling around -25°C is due to cooling of the environment rather than to the cessation of ice formation.

10. References

[1] Fahy GM. Analysis of "solution effects" injury: equations for calculating phase diagram information for the ternary systems NaCl-dimethylsulfoxide-water and NaCl-glycerol-water. Biophysical Journal. 1980;32:837-50.