Case Report A-3434

By Linda Chamberlain

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-3434 was a 90-year-old member with neuro cryopreservation arrangements who had kidney problems and a metastasized malignant neoplasm of the bladder. The member was pronounced legally deceased in California at 02:08 hrs on T-0 days in February 2022. The cause of death per the death certificate was respiratory arrest subsequent to cardiac arrest, with an underlying cause of malignant neoplasm of the bladder.

After stabilization and Field Cryoprotection (FCP), the patient was air transported to Alcor for cryogenic cooldown. The patient arrived at Alcor on T+1 days at 20:35 hrs. The cryogenic cooldown was initiated on T+1 days at 21:36 hrs and terminated on T+4 days at 20:42 hrs. CT scans were made of the patient's brain on T+52 days; the patient was then transferred to long-term maintenance at liquid nitrogen temperature.

Patient Assessment and Pre-Deployment

T-99 days

This was a new member with terminal bladder cancer that had metastasized to multiple organs and was going through a second round of chemotherapy. The member's height was 6'1" and weight was estimated to be 73 kg to 77 kg. Hospice care was not available because the member was still receiving medical treatment. As the member was on the Alcor Watch List, Alcor's Medical Response Director (MRD) called for a weekly update and learned that the member was in the hospital for a serious infection in the lower quadrants. Physicians also found a nondescript problem with the kidneys. There was no indication that the member would decline acutely. Alcor's MRD continued weekly calls with the member with no significant health changes.

T-36 days

The member successfully had kidney stents placed bilaterally, had been receiving frequent blood transfusions, and was experiencing increasing weakness.

T-29 days

The member was taken by ambulance to a hospital due to a fall at home. The member needed a blood transfusion, but critical shortages of blood due to the COVID-19 pandemic prevented this from happening. Additionally, the abdomen was distended.

The member had a hemoglobin count of 5.5 and had received 2 units of blood. A third unit was to be given the next day. A CT scan of the abdomen showed an impacted bowel. A discussion was held with the member about the potential for a delayed pronouncement of legal death while in in-home hospice care. The MRD and Alcor's surgeon consulted and agreed that, based on current information, a deployment was not yet justified.

T-24 days

The member received a blood transfusion, but his hemoglobin count continued to fall, there was some bleeding (location not known) and an unspecified infection (the COVID protocols made it difficult to get information, even family visits were limited). The physicians had requested a meeting with the member and the family for a palliative care discussion.

T-19 days

The member was discharged from the hospital. Despite repeated conversations regarding hospice, the member still had not contacted any hospice facilities. At 13:56 hrs the member called Alcor's MRD to report feeling weak and was enroute to the hospital to receive another transfusion. The member had a temperature of 39°C and at-home COVID tests showed that both the member and spouse were positive for COVID-19. The hospital had been clear that care options were limited, and the member needed to seriously consider hospice.

Suspended Animation, one of Alcor's strategic partners for standby, stabilization and transport (SST) was located near the member and a paramedic from a second strategic partner, International Cryomedicine Experts (ICE) was also in the area, so there would be immediate assistance in case of emergent need.

T-6 days

The member was now enrolled in in-home hospice care and receiving a home visit every three days or as needed depending on the member's condition. Hospice personnel agreed to inform Alcor of any changes, and as the member declined, they would schedule visits more often.

Preparation and Deployment

T-2 days

As the member had experienced a noteworthy decline over the last two days, the member's family called Alcor while the hospice nurse was at the home. The member was significantly less alert, in constant pain, and could no longer swallow pills. Regarding nutrition, the member had eaten two spoons of egg whites the day before and had half of an Ensure drink, but nothing this day outside of sips of water. The member was on Norco and sublingual morphine for pain.

There was almost no urine output. The vital signs taken by the hospice nurse were stable with a blood pressure (BP) of 128/78, heart rate (HR) of 105/min, and a capillary oxygen saturation (SpO_2) of 92% on room air.

Because this case was going to be a field cryoprotection, ICE was officially deployed for SST at 15:13 hrs. The ICE team was in the area and arrived at the member's home at 17:11 hrs. The member was alert and talking, but not oriented.

Standby and Stabilization

T-1 days

The hospice nurse called Alcor at 17:19 hrs and reported that the member had excessive secretions but the ability to swallow was limited. There had been little consumption of food or liquids all day. The member had received 10 mg of sublingual morphine twice that day. The nurse confirmed that during daytime hours she would be called to pronounce legal death. The after-hours line would be called for declaration after 17:00 hrs.

At 21:45 hrs the member had increased difficulty with breathing. The member's vital signs at 23:03 hrs were SpO_2 of 67%, HR 124/min, and 30 labored respirations per minute.

T-0 days

ICE was called by the member's family at 01:43 hrs to report that the member may have gone into cardiac arrest; this time is used in this report for the time of estimated cardiac arrest.

The death certificate shows the time of death as 01:08 hrs (PST). For this report, the time on the death certificate will be used as the time of pronouncement of legal death and converted to MST (02:08 hrs). ICE arrived at the patient's home and did not initiate stabilization procedures until the arrival of the hospice nurse and the legal declaration of death (see the Discussion section).

To initiate cooling, at 02:20 hrs manual cardiopulmonary support was started while the ROSC-U device was set up and initiated. The patient was placed into a portable ice bath (PIB) made from a body bag and 120 lbs. of ice were placed around the patient (see Discussion section). The first intraosseous device (B.I.G., Bone Injection Gun) was placed in the tibial tuberosity of the right leg at 02:20 hrs to access the vasculature for administration of the stabilization medications which were initiated at 02:22 hrs (see the below Table of Medications Administered for the names of the medications). A second intraosseous device was placed in the tibial tuberosity of the left leg at 02:34 hrs to allow the medications to be delivered more quickly. A King airway was inserted; however, the balloon cuff malfunctioned and had to be removed. Without the cuff inflated, air would be free to enter the stomach and fluids could enter the airway. Therefore, no airway was used, and cardiopulmonary support proceeded without ventilation.

Cardiopulmonary support was terminated at 04:00 hrs. The HOBO datalogger did not function. As the kit had two dataloggers, the second device was used and functioned properly (see the Discussion section). After stabilization, the patient departed the residence at 04:17 hrs and arrived at the funeral home for Field Cryoprotection (FCP) at 04:54 hrs. Cooling and chest compressions continued while waiting for the transport company to arrive.

Field Surgery and Cryoprotectant Perfusion

Field surgery was initiated at 05:14 hrs. The right carotid artery was cannulated at 05:28 hrs and the left carotid artery was cannulated at 05:41 hrs. Both burr holes were completed at 05:43 hrs and cephalic isolation was initiated at 05:48 hrs and completed at 05:52 hrs. The cephalon weighed 4.7 kg, prior to cryoprotection. Fluid return was noted from the vertebral arteries, suggesting that the Circle of Willis was intact.

Bladder #1 containing B1 solution without cryoprotectant was used at 06:00 hrs to prime the tubing circuit. All refractive index (RI) readings were taken from the seepage emanating from the jugular veins. The refractive index readings were inconsistent. This resulted in only recording a refractive index reading once, at the start of the 1-hour countdown to termination of cryoprotectant perfusion (see the Discussion section).

Pressure gauges are not currently in the kit as pressure is estimated by the height of the bladders on the pole (see the Discussion section). The teeter-totter device that allows a smooth mixing of different solutions of perfusate was found to be broken, so bladders were hung one at a time (see the Discussion section for a more detailed explanation).

The gravity-induced perfusion flow was initiated at 06:16 hrs with Bladder #2 containing nM22 cryoprotectant with a concentration of 0.05 CNV. See the below Table of Concentrations (Brix) of nM22 solution for the precalculated refractive index of the individual bladders, and the refractive index of the effluent samples.

The refractive index of the effluent was 50.3 Brix at 09:05 hrs (see the Discussion section); the 1-hour countdown to termination of cryoprotectant perfusion was started.

Per the cryoprotection protocol, the ramp is to be paused at 30 Brix (50% of the desired terminal concentration) to allow the patient to come to osmotic equilibrium. When the bladder system is used, bladders 6 & 7 represent the pause. The cephalic 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 the terminal concentration is obtained.

Field cryoprotection ended at 10:08 hrs. The refractive index was 50.3 Brix. Perfusion was terminated at 10:10 hrs. The cephalon weighed 4.9 kg at 10:15 hrs, after cryoprotection. This was a weight gain of 0.2 kg, or 4 percent.

Patient Transport

The patient was placed in a neuro shipping container and covered with approximately 5 lbs. of dry ice at 10:20 hrs. The temperatures at 10:25 hrs were nasopharyngeal (NPT): 3°C; burr hole (BHT): 0.4°C; arterial line: 1°C. The data logger showed an isotherm at -22.6°C.

An isotherm (freezing event) is a period of interrupted temperature descent observed on the time vs. temperature graph of a specimen as the specimen undergoes a phase transition, for example when freezing a liquid to a solid. An isotherm occurs as energy is exchanged to rearrange molecules into the new phase, instead of changing the temperature of the system. In the context of cryonics, an isotherm is undesirable because it is an indicator of ice formation, and therefore incomplete vitrification. The formation of a glassy solid by vitrification, which involves no crystallization, does not express an isotherm.

As a result of working with little sleep, ICE personnel slept for a few hours before driving the patient to Alcor. The patient departed California on T-0 days at 15:30 hrs and arrived at Alcor on T-0 days at 20:35 hrs. The BHT was -79°C upon arrival at Alcor (see the Discussion section).

Cooling to Liquid Nitrogen Temperature

A computer program was used to initiate cryogenic cooldown at 21:36 hrs on T-0 days, plunging to -110°C and descending thereafter at -1°C/hour to liquid nitrogen temperature. At 21:37 hrs neither the primary nor the backup cooldown systems showed any temperature readings. A back-up computer was successfully installed, and cooldown continued (see the Discussion section).

Cooldown was terminated at 20:42 hrs on T+4 days. On T+52 days, CT scans were made of the patient's cephalon, and the patient was then transferred to long-term maintenance at liquid nitrogen temperature.

Timeline and Time Summaries

TIMELINE

T-0 days	01:43	Estimated time of cardiac arrest
T-0 days	02:08	Pronouncement of legal death
T-0 days	02:20	Start ice bath cooling
T-0 days	02:20	Insert Nasopharyngeal probes (estimated,
T-0 days	02:20	Start mechanical chest compression
T-0 days	02:20	Placement of first intraosseous device (IO)
T-0 days	02:22	Admin of first medication (20 g, sodium citrate)
T-0 days	02:34	Placement of second intraosseous device (IO)
T-0 days	02:49	Admin of final medication (200 ml, Decaglycerol/THAM)
T-0 days	04:00	Stop cardiopulmonary support (NPT not recorded)
T-0 days	04:17	Start transport of patient to place of surgery/cryoprotection (funeral home)
T-0 days	04:54	Arrived at funeral home
T-0 days	05:14	Start of field surgery
T-0 days	05:18	Start of cephalic isolation
T-0 days	05:50	End of surgery/cephalic isolation (estimate)
T-0 days	05:52	Weight of cephalon pre-perfusion (4.7 kg)
T-0 days	06:16	Start of open-circuit bladder cryoprotection
T-0 days	10:08	End cryoprotection (final concentration = 51.1 Brix)
T-0 days	10:15	Weight of cephalon post-perfusion (4.9 kg, 4% gain)
T-0 days	10:20	Start of dry ice cooling (NPT =37°C, BHT = 33°C)
T-0 days	15:30	Begin vehicle transport of patient to Alcor
T-0 days	20:35	Arrival of patient at Alcor (see Discussion re temp)
T-0 days	21:36	Start patient cryogenic cooldown
T+4 days	20:42	End of cryogenic cooldown
T+51 days	00:00	CT scans at LN2 temperature
T+51 days	00:00	Transfer of patient to long-term maintenance

TIME SUMMARIES

Event Duration (hr:min)		Days	Time	
STABILIZATION				
00:25	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	02:08	Pronouncement of legal death
00:37	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	02:20	Start mechanical chest compression
00:39	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	02:22	Admin of first medication (20 g, sodium citrate)
00:27	From:	T-0	02:22	Admin of first medication (20 g, sodium citrate)
	Till:	T-0	02:49	Admin of final medication (200 ml, Decaglycerol/THAM)
FIELD SURGERY AND CRYOPROTECTANT PERFUSION (FCP)				
03:31	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	05:14	Start field surgery
00:36	From:	T-0	05:14	Start field surgery
	Till:	T-0	05:50	End of surgery/ cephalic isolation (estimate)
04:33	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	06:16	Start of open-circuit bladder cryoprotection
03:52	From:	T-0	06:16	Start of open-circuit bladder cryoprotection
	Till:	T-0	10:08	End cryoprotection (final concentration = 51.1 Brix)
08:25	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	10:08	End cryoprotection (final concentration = 51.1 Brix)
00:36	From:	T-0	05:14	Start field surgery
	Till:	T-0	05:50	End of surgery/ cephalic isolation (estimate)
01:02	From:	T-0	05:14	Start field surgery
	Till:	T-0	06:16	Start of open-circuit bladder cryoprotection
04:54	From:	T-0	05:14	Start field surgery
	Till:	T-0	10:08	End cryoprotection (final concentration = 51.1 Brix)
DRY ICE AND LIQUID NITROGEN COOLDOWN				
00:12	From:	T-0	10:08	End cryoprotection (final concentration = 51.1 Brix)
	Till:	T-0	10:20	Start of dry ice cooling (NPT =37°C, BHT = 33°C)
08:37	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	10:20	Start of dry ice cooling (NPT =37°C, BHT = 33°C)
18:52	From:	T-0	01:43	Estimated time of cardiac arrest
	Till:	T-0	20:35	Arrival of patient at Alcor (see Discussion re temperature)
01:01	From:	T-0	20:35	Arrival of patient at Alcor (see Discussion re temperature)
	Till:	T-0	21:36	Start patient cryogenic cooldown

Table of Medications Administered

T-0 DAYS

Time of admin not available

MEDICATION	DOSE	PURPOSE
Propofol	200 mg	Anesthetic; reduces cerebral metabolic demand; reduces the theoretic possibility of increased awareness during aggressive CPS.
Sodium citrate	10 - 20 g	Anticoagulant; prevents blood clot formation.
Heparin	50,000 IU	Anticoagulant; prevents blood clot formation.

Vasopressin	80 IU total	Vasopressor; increases blood pressure during CPS.
Minocycline	200 mg	Antibiotic and neuroprotectant
SMT (S-methyl-isothiourea)	400 mg	Neuroprotectant (iNOS inhibitor); protects the brain from ischemic injury; raises blood pressure.
Vital Oxy (w/ saline)	70 mL max	Antioxidants: melatonin, vitamin E (D-alpha tocopherol), PBN (alpha Phenyl t-Butyl Nitrone) and anti-inflammatory carprofen.
Decaglycerol/ THAM	200 ml	Decaglycerol inhibits cerebral edema.
Streptokinase	25,000 IU	Added to FCP Bladder #1

Notes:

1. The videos of this stabilization were lost. Since the field report is written using the information from the videos, there are no medication administration times for this report. The table above shows the medications and the standard dosages that are on the Full Stabilization Medications Protocol.

2. No antacid was administered because the patient was not intubated.

Table of Concentrations (Brix) of nM22 Solution

A-3434 step-ramp, nM22						
Preferred endpoint is effluent over 49.9 Brix for 1/2 hr						
2-liter bag labeled	[nM22], CNV	Brix (calc)	bag started, hr:min MST	bag started, hr:min post-pro-nouncement	bag flow rate, ml/min	effluent, Brix
1	0.00	9.2	5:00	3:42		
2	0.05	11.8	5:16	3:58	125	
3	0.08	13.1	5:35	4:17	95	
4	0.14	15.3	5:52	4:34	118	
5	0.23	19.0	6:17	4:59	80	
6	0.50	29.9	6:32	5:14	133	
7	0.5	29.9	6:49	5:31	118	
8	1.06	52.3	7:04	5:46	133	
9	1.06	52.3	7:22	6:04	111	
10	1.06	52.3	7:36	6:18	143	
11	1.06	52.3	7:50	6:32	143	
12	1.06	52.3	8:05	6:47	133	50.3
13	1.06	52.3	8:28	7:10	87	
14	1.06	52.3	8:49	7:31	95	
END			9:10	7:52	95	50.3

Note: The bladders of pre-mixed concentrations of cryoprotectant are made up in advance and kept on hand. At the time the bladders used on this case were made up the protocol was to have bladder #1 contain only B1 washout solution. It has been learned on recent cases that starting perfusion with a low concentration of cryoprotectant, and not just washout solution, mitigates developing edema in the patient. For this reason, the protocol is now to always start with bladder #2 which does contain cryoprotectant. Since there is still a stock of bladders where there is a bladder marked #1, those perfusions are noted as having been initiated with bladder #2.

Discussion

Standby, Stabilization and Transport

The member's family reported at 01:43 hrs that the member may have gone into cardiac arrest; this time is used in this report for the time of estimated cardiac arrest. ICE instructed the family to notify the on-call hospice nurse. Three members of the ICE team arrived at the member's home at 02:06 hrs. The hospice nurse arrived at 02:18 hrs. The ICE team waited for the hospice nurse to arrive and noted the time of arrival. However, the nurse gave a different time for pronouncement; she stated the time of death would be the time she arrived at the house. When questioned about the accuracy of the time, she stated she was not going to change the time of death.

The videos of this stabilization were lost. ICE personnel do not know how it happened and will be diligent in the future to not let this happen again.

On field cryoprotection cases, Alcor supplies a kit containing a body bag to be used as the portable ice bath. The team placed the ice around the patient but planned to add the water and the SCCD once the body bag was placed at the funeral home because (1) the water would have been too heavy, and (2) the funeral home personnel were not cooperative and would not wait for the water or the SCCD to be added. Given the cooling rate of this patient, and the relatively poor CT scans, initial cooling of this patient was inferior to what could have been achieved with a regular portable ice bath and squid. This body-bag usage policy may need to be revisited.

The balloon cuff on the King airway malfunctioned. Therefore,

no airway was used, and CPS proceeded without ventilation. A new airway was placed in the kit when it was prepared for the next case.

The HOBO datalogger did not function. The patient's home was small and crowded. The stabilization kits had to be placed outside on the porch and the surgical and perfusion kits stayed in the rental vehicle on the street, about a block away. While placing the nasopharyngeal probe during the stabilization, the HOBO data logger would not turn on. The team was not aware of the second datalogger in the kit and circumstances did allow time to search for it. For future cases, the team should review availability of spare equipment in the field kit. Temperature probes should be placed in at least two locations (for example, rectal and tympanic) for redundancy.

To further complicate matters, the funeral home personnel were uncooperative and impatient. They had been told that it would take approximately 45 minutes to prep the patient before they could remove the patient and start the transport to the funeral home. They arrived approximately 30 minutes early and were not only annoyed they would have to wait, but they said that they were going to leave, and the cryonics team would have to call them again later, after preparations were finished, causing a significant delay. Additional time and effort went into convincing them to stay. Fortunately, two team members continued to stabilize the patient and, after arrival at the funeral home, a second data logger was found and did function properly.

The burr hole temperature probe was accidentally dislodged from the connector. Field repair to the probe was made. No screwdriver kit was available but surgical tools were used to reestablish the connection.

The burr hole temperature was -79°C upon arrival at Alcor but the burr hole probe had slipped out of the patient upon opening the body bag. It is possible the probe was no longer situated in the burr hole upon arrival.

Field Cryoprotection

The effluent refractive index (RI) readings taken from the jugular vein were not as consistent as experienced on previous cases. To see if the refractometer was malfunctioning, samples were taken from the bladder because the RI of each bladder is always shown on the label. The readings still varied greater than normally expected despite the refractometer being calibrated several times. This resulted in only taking a refractive index reading once, at the start of the 1-hour countdown to termination of cryoprotectant perfusion. To determine the final hour of perfusion, several measurements were taken, and an average (50.3 Brix) was used to determine if the perfusion should be terminated. Alcor personnel checked the refractometer but found it to be functioning normally. The refractometer in the kit has been replaced in case the malfunction was intermittent.

The kit no longer contains a pressure gauge for determining perfusion pressure because it would routinely cause the commercial data loggers to short out and result in loss of data. The pressure transducer devices Alcor uses were built in house to simulate the input of a thermocouple amplifier by generating a linearly related voltage as a function of pressure. This prevents the team from being bogged down with monitoring equipment. With the switch to HOBO loggers these started to feed back into the device in a way that would damage them. Alcor staff elected to remove that component.

Until the Universal Data Logger (UDL) being developed at Alcor is available, the field teams will need to estimate the perfusion pressure by the measured height of the bladders. The estimated height of the bladders on the pole was 36" to 38" which is (36" x 2.054 mmHg per inch of height = 74 to 78 mmHg maximum arterial pressure at the infusion site). The goal is to have the pressure between 70 and 80 mmHg and the bladders can be raised or lowered as needed to optimize flow and for protection of the vasculature.

The gravity feed system for FCP uses a tripod that can be adjusted for height to control the arterial pressure. The pre-mixed cryoprotectant was in a series of bladders with graduated concentrations [measured by the refractive index (RI) in Brix units]. By hanging two bladders with different RI concentrations on a teeter-totter atop the tripod, the bladder with the lower RI runs out and becomes lighter. At the mid-way point, the teeter-totter will allow both bladders to flow, mixing the two concentrations and creating a smoother transition from one concentration to the next. When the bladder with the lower RI runs out, the full concentration of the bladder with higher RI is then flowing exclusively. This process allows for a smoother curve in the increasing concentrations of cryoprotectant.

Unfortunately, the teeter-totter device broke resulting in the loss of the mixing function. Bags were infused one at a time. A new, sturdier design for the teeter-totter device has been developed and will be included in the kits going forward.

Cryogenic Cooldown

At the initiation of cooldown an issue developed both in the primary and the back-up cooldown cart. Neither system showed any temperature readings. An additional back-up computer was successfully installed on the cooldown cart. The cause of this problem was not isolated, but it is possible that a software conflict prevented the temperature module from operating correctly.

S-MIX and CT Scans

The total normothermic equivalent ischemic exposure time of this patient was 04:02 hours, which is a relatively high value for a case with SST. As can be seen in the CT scan below most parts of the brain did not receive the minimum concentration of cryoprotectant to suppress ice formation. The CT scan also does not show CPA-

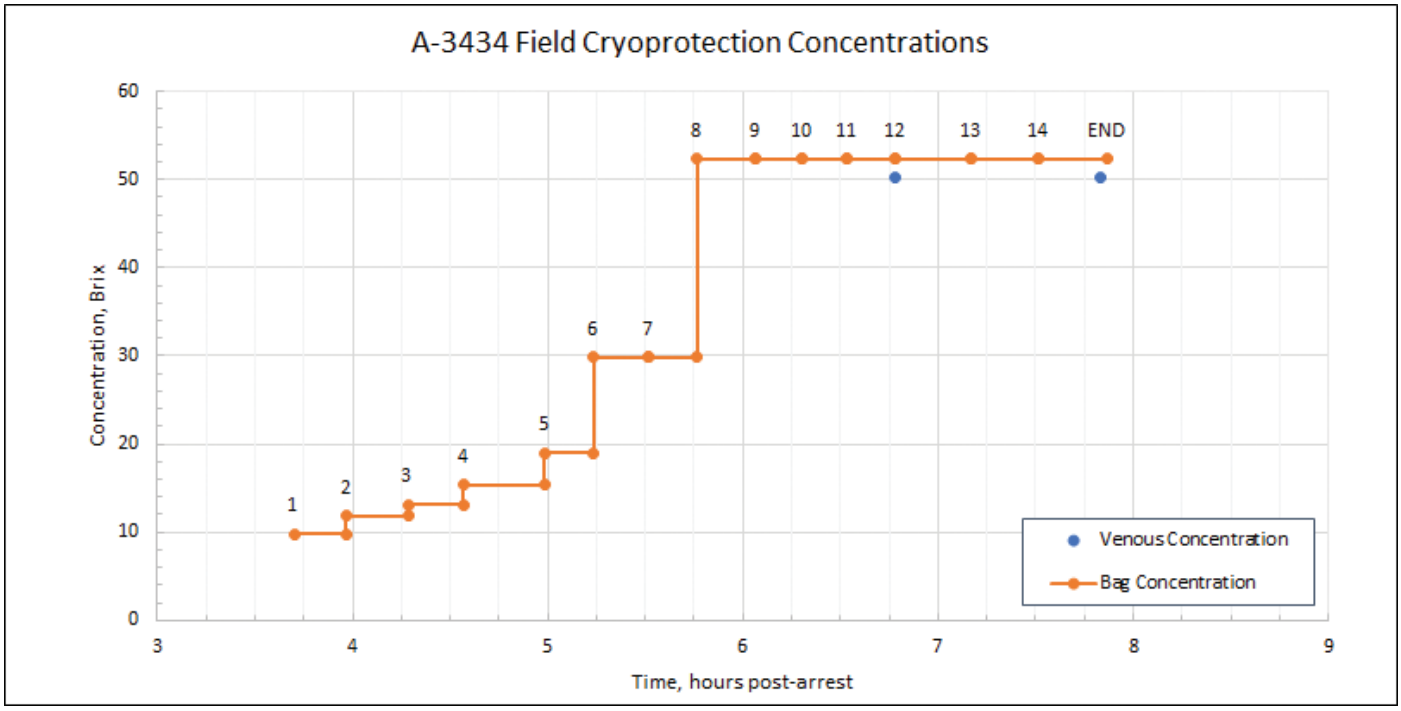
induced shrinking, which further corroborates that the patient suffered significant ischemic injury prior to cryoprotection.

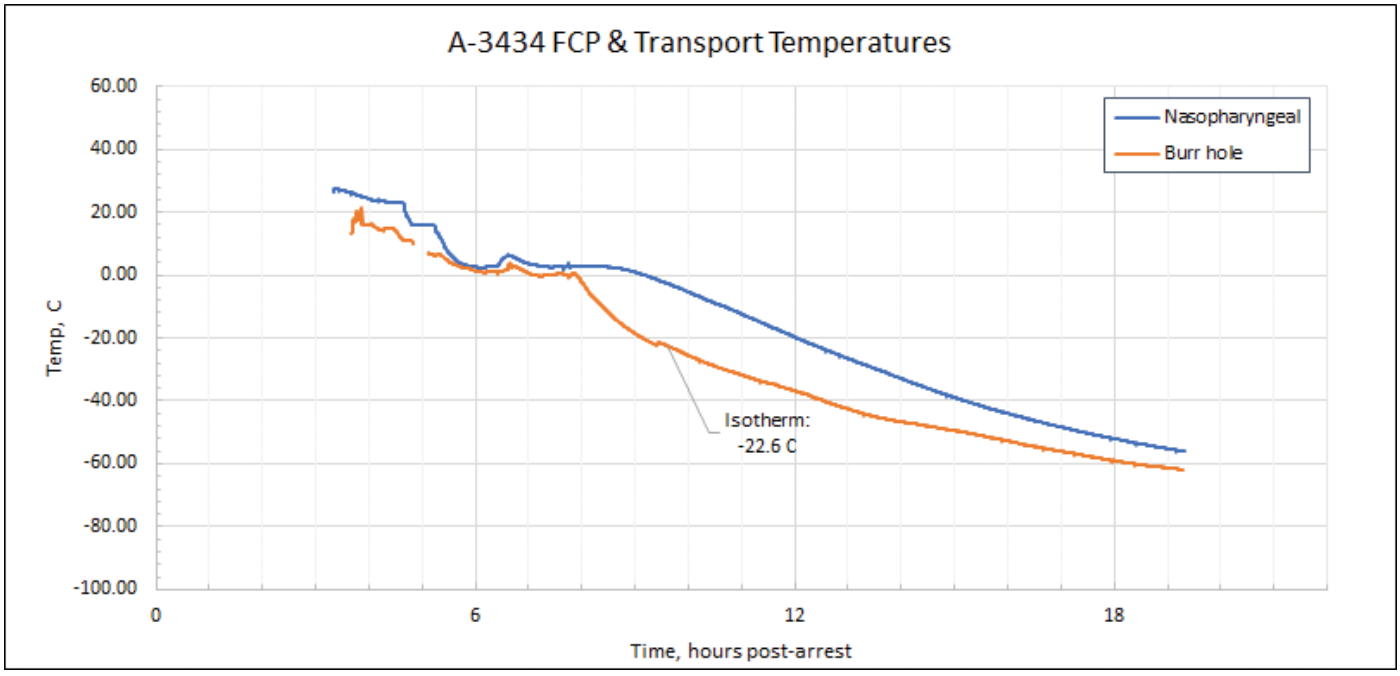
The most plausible explanation for the poorer CT scan results is that the patient spent a significant period of time at normothermic temperatures prior to the start of procedures, ventilation was omitted during cardiopulmonary support, and initial cooling was poor.

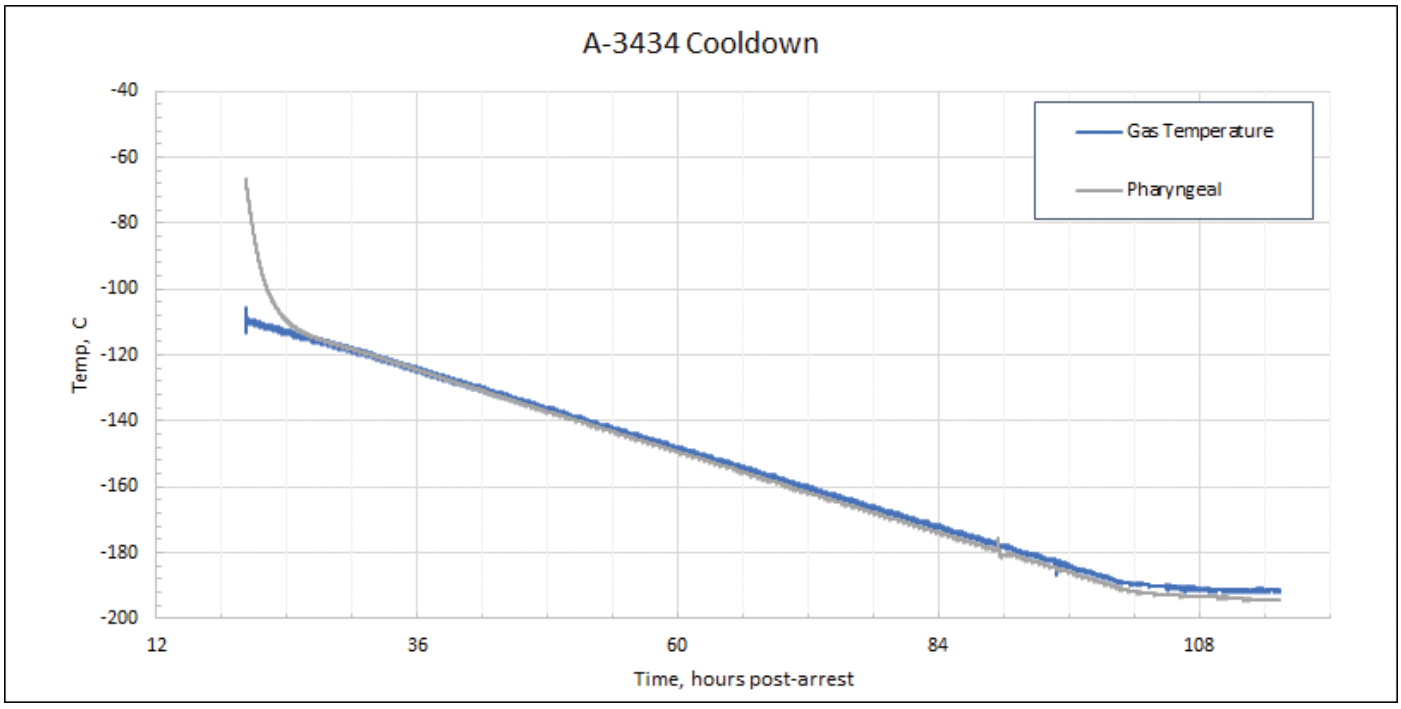
S-MIX

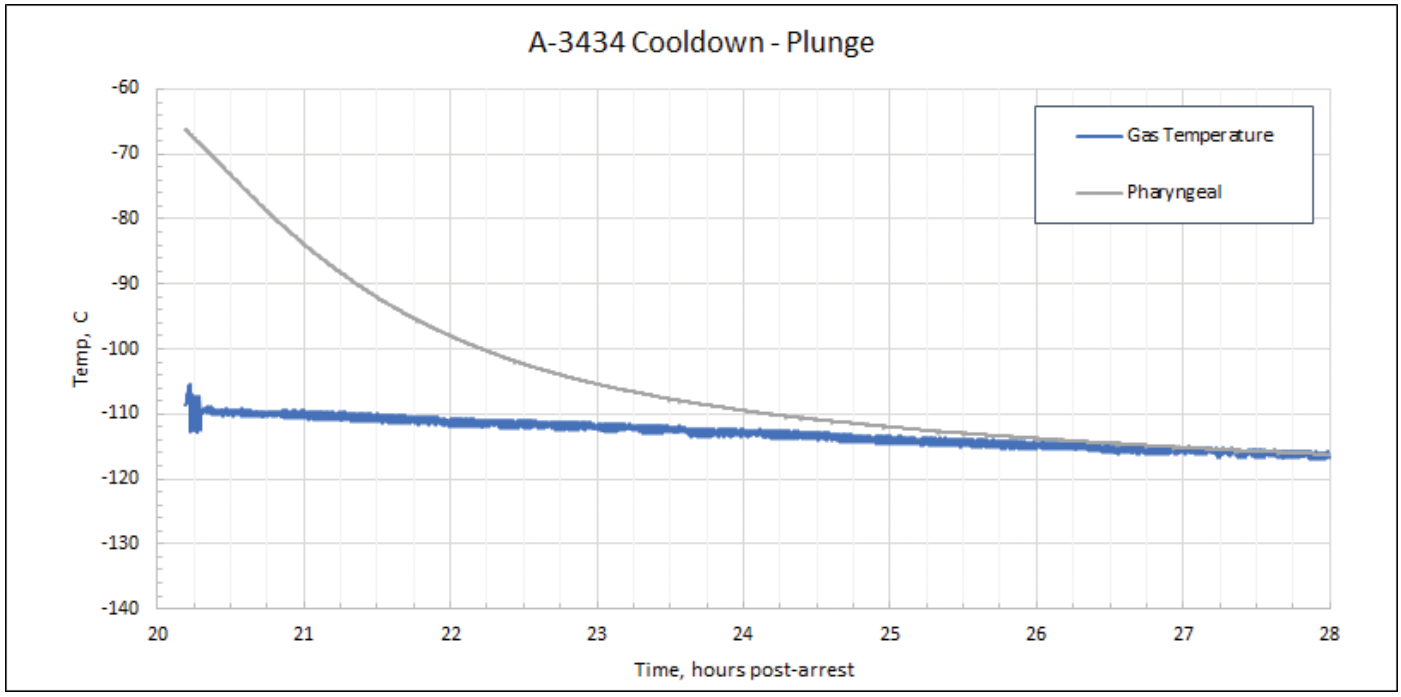
The Standardized Measure of Ischemic Exposure (S-MIX) expresses the total ischemic exposure prior to the start of cryogenic cooling as the equivalent duration of normothermic ischemia. An S-MIX of 00:00 (hh:mm) is the ideal case of no ischemic damage. The higher the S-MIX time, the more damage.

Cryoprotection and Temperature Graphs





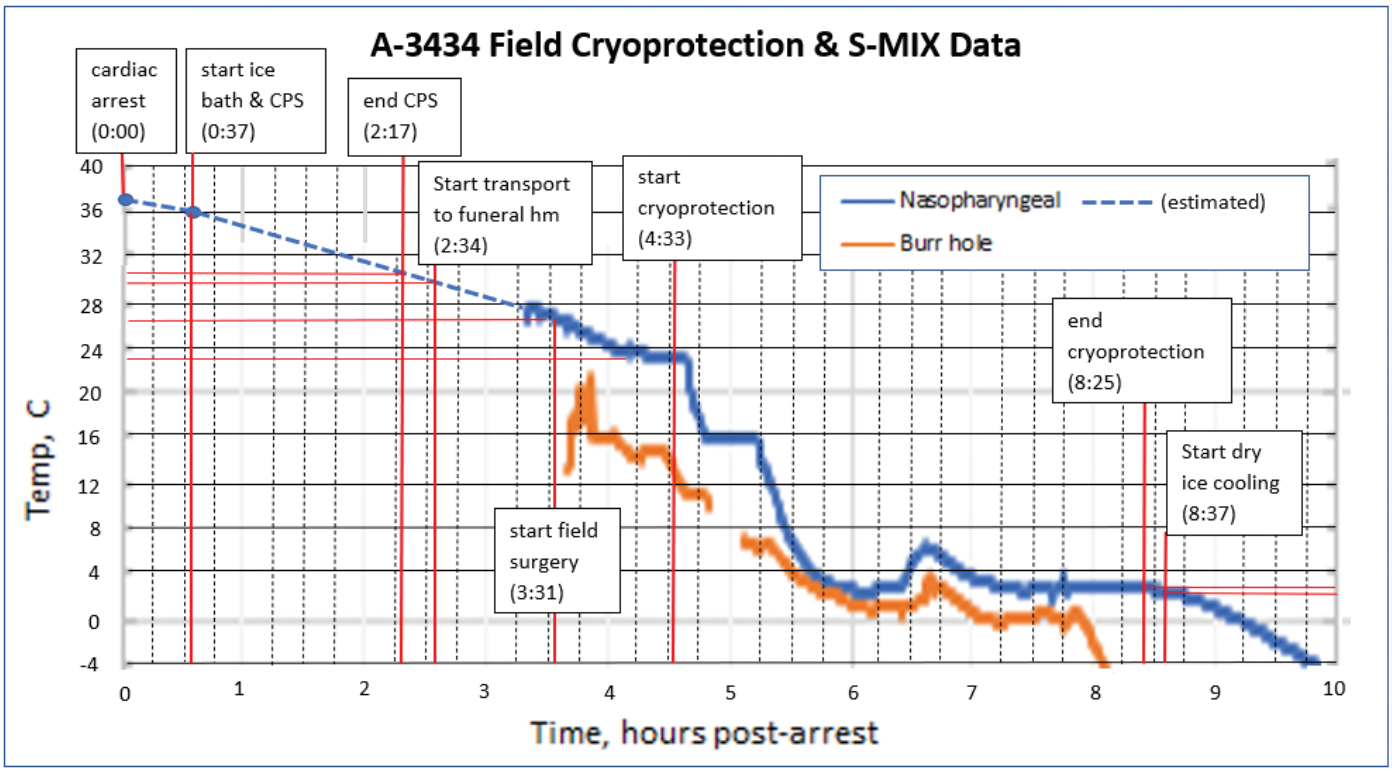




Factors that improve the S-MIX, and that are quantitatively accounted for in the below table are: shorter times at higher temperatures, ventilation during cardiopulmonary support (CPS), and oxygenation during blood washout. As calculated below, S-MIX duration for this case is 04:02 hrs.

CT Scans

The post-cryogenic cooldown CT scan was obtained on T+51 days; the patient was at liquid nitrogen temperature (-196°C).



event	segment number	days (T+X)	time (hh:mm)	post-arrest (hh:mm)	T_{naso} (deg C)	CPS with ventilation	washout oxygenation	S-MIX (hh:mm)
circulatory arrest		T-0	01:43	00:00	37.0			
	seg 1		00:37	00:37	-1.0	no	no	00:36
start of ice bath cooling & CPS		T-0	02:20	00:37	36.0			
	seg 2		01:40	01:40	-5.2	no	no	01:19
end CPS		T-0	04:00	02:17	30.8			
	seg 3		00:17	00:17	-0.9	no	no	00:11
start transport to funeral home		T-0	04:17	02:34	29.9			
	seg 4		00:57	00:57	-3.2	no	no	00:31
start field surgery		T-0	05:14	03:31	26.7			
	seg 5		01:02	01:02	-3.6	no	no	00:27
start open-circuit cryoprotection		T-0	06:16	04:33	23.1			
	seg 6		03:52	03:52	-20.1	no	no	00:51
end cryoprotection		T-0	10:08	08:25	3.0			
	seg 7		00:12	00:12	-0.7	no	no	00:01
start dry ice cooling		T-0	10:20	08:37	2.3			
	seg 8		01:13	01:13	-2.3	no	no	00:06
temperature drops to 0 deg C		T-0	11:33	09:50	0.0			
totals:			9:50	9:50	-37.0			04:02

The CT scans show a heterogenous distribution of frozen blood and areas with sub-optimal concentrations of M22. Only a few minor areas show evidence of concentrations of M22 necessary for vitrification. ■

Cryoprotectant Distribution (Post-cryopreservation CT scan)