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CRYOPRESERVATION OF KIM SUOZZI

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CRYOPRESERVATION OF KIM SUOZZI (PATIENT A-2643)

By Chana de Wolf

INTRODUCTION

The last comprehensive review of Alcor's current cryopreservation protocol, including a detailed description of changes since 1990, was provided in the case report for Patient A-1097 (2007). The only change in neuropreservation protocol since 2007 is the removal of a cold carotid flush to selectively cool the head prior to cephalic isolation (which was a one-time experiment). The current protocol is to cool the entire patient to a temperature compatible with cessation of CPS prior to cephalic isolation and cannulation of the carotids for subsequent washout and cryoprotection. The standard protocol was used in the current case.

MEMBERSHIP APPROVAL

Kim completed membership documents, including Consent for Cryopreservation, Last Will and Testament of Human Remains (Authorization of Anatomical Donation), Emergency Standby Provisions, and a Cryopreservation Agreement between Kim Suozzi and Alcor Life Extension Foundation on October 12, 2012. The agreement was approved by the Board of Directors and executed on October 31, 2012, due to special circumstances related to the charitable funding of this case.

MEDICAL HISTORY

Born June 10, 1989, Kim Suozzi was a 21 year old college student when she experienced a focal seizure in March 2011. Follow-up revealed a high grade malignancy and surgery was scheduled immediately and performed five days later. The post-surgical pathology report confirmed the presence of (Grade IV) Glioblastoma multiforme (GBM), a type of malignant brain tumor,

in the left frontoparietal area. This tumor was removed during surgery and Kim was monitored for recurrence afterward. A local recurrence (in the same location) would have indicated another surgical resection. Unfortunately, recurrence occurred 10 cm from the original tumor site, in the left cerebral peduncle of the brain stem, rendering the tumor inoperable.

Kim and her power of attorney focused their efforts on curing her cancer, but decided that if that did not work they would preserve her brain, as much as possible, from the advancing tumor. Several additional experimental treatments were undertaken by Kim over the following 22 months. Unfortunately, Alcor is not in possession of detailed records regarding the number, type, or efficacy of these treatments, but ultimately, they were all unsuccessful. In early January 2013 Kim attempted to gain access to a new clinical trial but was denied, spurring her decision to come to Scottsdale to enter hospice care near Alcor.

A physician's visit in St. Louis, Missouri, on January 3, 2013, noted normal vital signs and the ability to walk short distances with assistance, but that Kim suffered from periods of confusion and could only respond to questions with "yes" or "no." She was approved for travel to Arizona.

On January 4, 2013, Alcor received an emergency text at 01:11 that Kim was "near death" and planned to travel by airplane to Phoenix that day. Upon contacting Kim's boyfriend, Alcor's Medical Response Director found that the situation wasn't quite as dire as it sounded, but that they had lost his direct phone number and decided to call the emergency number to make contact with Alcor as soon as possible.

AGONAL PHASE

Kim arrived in Phoenix with her boyfriend (who was also her medical power of attorney) on the morning of January 4, 2013, and checked into a local hotel to await hospice evaluation. Alcor's Medical Response Director visited that evening and observed that Kim was unable to communicate verbally and could only respond to very simple questions by nodding her head. She indicated that she had had a continuous low grade headache for several days and other pain, but could not specify where.

A hospice nurse arrived during Alcor's Medical Response Director's visit to announce that Kim was approved for hospice inpatient admission. This was surprising news because the hospice had not yet performed her intake evaluation nor received her medical history. The nurse took Kim's vital signs before leaving, which were normal.

Kim was admitted to hospice on January 5, 2013. Upon visiting later that day, Alcor's Medical Response Director noted her condition to be greatly improved compared to his observation the day before. She was alert, sitting up and speaking full sentences in conversation with visitors. She was unable to recall the previous day and did not remember meeting Alcor's Medical Response Director or the hospice intake nurse. She continued to experience headaches that were not alleviated by medication. She enjoyed her last food and drinking fluids on this date.

The hospice medical director examined Kim on January 7, 2013. Her vital signs were normal and she was alert but not as talkative as she was upon admission. His opinion was that, based on her current

condition, she could live for quite a while longer and that the ability to keep her in hospice would depend on how strictly she would adhere to her decision to voluntarily stop eating and drinking (VSED).

Physical assessments continued in hospice as Kim experienced an alternation of “good days” and “bad days.” A good day consisted of texting on her phone, playing video games, petting her cat, and limited verbal communication. A bad day was one spent sleeping and suffering from an intractable headache. After one week in hospice without food or drink, Kim’s family was advised that she would soon be discharged until she became more comatose. That same evening (January 12, 2013), Kim had a very restless night. The hospice delayed her discharge to allow the family time to find a suitable location.

Upon discharge and relocation to a condominium near Alcor on January 15, 2013, Kim appeared comatose and emaciated. Her blood pressure (102/68) and oxygen saturation (97%) were normal, but heart rate (112) was elevated and respiratory rate (9/min, with periods of apnea) was depressed. She showed obvious signs of dehydration, such as skin tenting, and weighed 90 pounds—a loss of 24 pounds in 10 days. Extremities were cool but no mottling of the skin was observed.

Nurses noted another significant change in Kim’s condition on January 16, 2013. Her heart rate had risen to 178 while blood pressure dropped to 92/70 and oxygen saturation to 92%. Respiratory rate remained depressed at 9/min with apnea and she was noted as completely unresponsive.

Kim’s family was awoken by a pulse oximeter alarm on the morning of January 17, 2013. Her boyfriend called the hospice at 05:54 to request a nurse. He reported that Kim’s breathing was erratic and observed what he later assumed to be her last breath during that call. He was advised that a nurse would arrive within 20 minutes, but the nurse did not arrive until 06:59. Death was pronounced at 07:00.

PREPARATION AND DEPLOYMENT

After her arrival in Scottsdale, Arizona, Alcor’s Medical Response Director

visited Kim frequently in order to monitor her condition and to provide ongoing updates to the stabilization team. After some transient improvement upon admission to the hospice, Kim afterward declined food and hydration. On January 16, hospice nurses notified Alcor to another significant change in Kim’s condition and stated that legal death was imminent.

Standby was formally initiated on January 16. Initially, three Alcor staff members were available for standby, with two additional volunteers en route to Arizona to be available the next day. The transport vehicle was prepared and brought on-site following a Deployment Committee meeting.

Alcor’s Medical Response Director received a call from Kim’s boyfriend at 05:57, immediately after he had called the hospice to request a nurse. He stated that Kim’s breathing was very erratic, but later assumed that she was in respiratory arrest at this time, since he had last observed a breath followed by complete relaxation at approximately 05:55.

Alcor’s Medical Response Director arrived at Kim’s location at 06:10 and the remaining stabilization, surgical, and cryoprotective perfusion team members were notified of the need to mobilize immediately. Another stabilization team member arrived at 06:25, and two more arrived at 06:45.

STABILIZATION

Because the stabilization team arrived before the hospice nurse, Kim’s family placed ice bags around her head and chest and allowed the team to prepare the ice bath, the Lucas 2 mechanical chest compression device, and medications on-site in anticipation of the nurse’s imminent arrival and pronouncement.

The hospice nurse did not arrive until 06:59—more than one hour after notification of Kim’s condition. Kim was pronounced at 07:00 and placed in the ice bath on top of a layer of ice and the Lucas positioned appropriately. Mechanical chest compressions were started at 07:03 and additional ice was added to the ice bath to surround Kim.

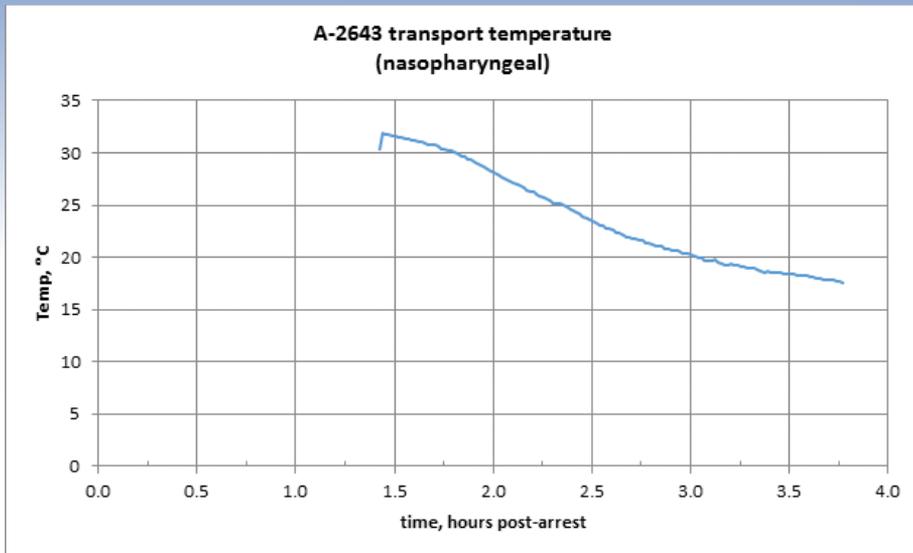
Two team members established IV access using the Bone Injection Gun and began pushing medications. The first medication, propofol, was administered at 07:06 via the left tibial (LT) line to reduce cerebral metabolism, immediately followed by streptokinase to dissolve existing blood clots. A second line was established in the right tibial (RT) plateau at 07:07 through which epinephrine was administered via a syringe pump beginning at 07:09. At the same time, administration of the VitalOxy neuroprotectant cocktail was begun via the LT line.

A secure airway was established with the King airway device at 07:13 and manual ventilations (6-8 per minute, room air) commenced with an Ambu bag valve mask. A nasopharyngeal thermocouple was placed in the left nostril at 07:19 and stapled in place to prevent accidental removal. The temperature data logger had been found to be inoperable and a team member left the room at 07:19 to look for batteries in the transport vehicle.

At 07:20 the Lucas 2 stopped working and a team member performed manual chest compressions while the battery was replaced. At 07:25 the Lucas was restarted and the data logger batteries were replaced. The data logger was set to collect nasopharyngeal temperature data once per minute. The initial left nasopharyngeal temperature reading at 07:25 was 31.4°C.

With all equipment now in working order, Kim was moved (within the PIB) from the condo at 07:28 and loaded onto the transport vehicle at 07:30. Administration of medications continued during transport. Upon arrival at Alcor at 07:45 a few medications still remained to be given, so Kim remained in the vehicle until administration was completed at 07:51. The Detailed Timeline box shows medications administered during the course of stabilization.

At 07:53 the PIB was unloaded from the transport vehicle and brought into the operating room. Mechanical chest compressions continued in order to further reduce temperature prior to cephalic isolation. Nasopharyngeal temperature was 29.0°C at 07:54. The SCCD was applied at 08:10.



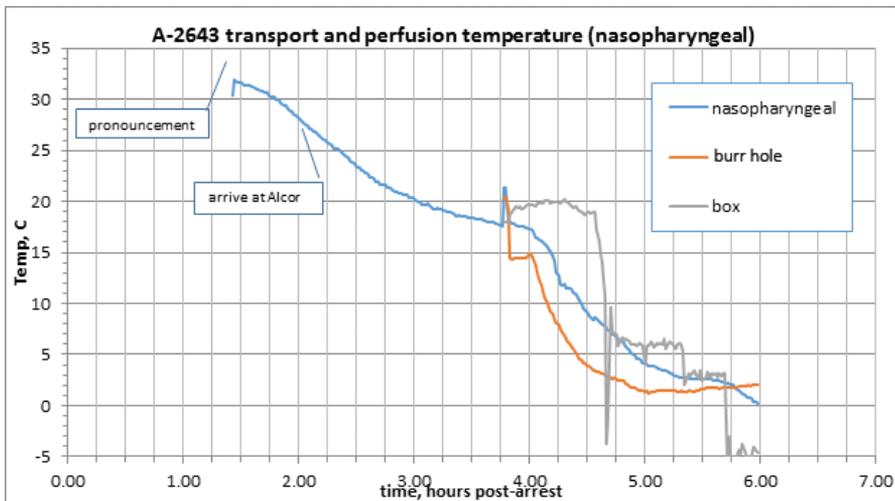
Appendix B: Transport temperature (nasopharyngeal)

SURGERY

Surgery commenced with Kim still in the PIB. As cardiopulmonary support continued, Kim's hair was clipped and the remaining hair shaved while ensuring that ice remained around the head. Upon completion of hair removal at 08:15, incisions were made for creating the left and right burr holes and skin was separated using a retractor to prepare the tissue for drilling of burr holes using a craniotome. Burr holes were completed at 08:22 and crack phone probes were inserted into each

burr hole and placed in contact with the surface of the brain at 08:36.

Further surgical procedures were initiated once Kim's temperature had dropped below 20°C. The right and left carotid arteries were isolated at 09:17 and 09:20, respectively, with Kim still in the PIB and receiving mechanical chest compressions. After consulting with a medical advisor regarding the appropriate temperature for cephalic isolation, Kim was removed from the PIB, placed on the OR table at 09:33 (nasopharyngeal temperature = 18.0°C),



Appendix C: Transport and perfusion temperature (nasopharyngeal)

and cephalic isolation was completed at 09:43.

At 09:45 the cephalon was placed in the holding ring of the neuroperfusion box and nasopharyngeal temperature data collection was switched from the handheld data logger to the main computer. The carotids were then cannulated with 18 Fr catheters. The perfusion circuit had already been primed at this time. The temperature of the washout perfusate (B1) was 3.0°C.

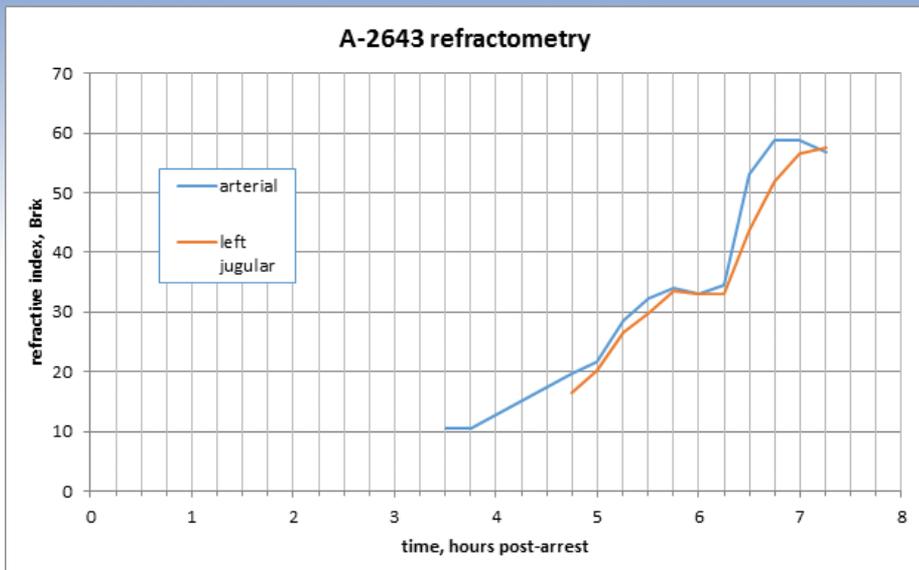
Flow was established at 09:59. The nasopharyngeal thermocouple read 17.2°C, while the burr hole thermocouple read 14.6°C.

CRYOPROTECTIVE PERFUSION

Washout of Kim's blood was started at 09:59. A total of 6 liters of B1 were perfused during washout. After washout the cryoprotectant ramp was started at 10:19 in order to gradually increase the concentration of M22 cryoprotectant into Kim. The washout intake line was closed, changing the system from "open circuit" perfusion to "closed circuit" perfusion (i.e., recirculation of cryoprotectant). The left jugular vein was then isolated but the right jugular vein could not be located. A thermocouple / sampling line was placed in the left jugular and connected to the thermocouple and process refractometers at 10:30.

Cooling in the cephalon enclosure started at 10:32. At this time, arterial temperature read 3.2°C, right venous temperature read 4.2°C, left venous temperature read 15.9°C, and nasopharyngeal temperature read 8.6°C. Eyeball shrinkage due to dehydration was noted at 10:57. Observation of the brain indicated greater retraction of the left hemisphere than the right.

Pump speed was gradually reduced to maintain pressure within an acceptable range (100-200 mmHg) as perfusate viscosity increased due to decreasing temperature and increasing cryoprotectant concentration. At 11:41 the cryoprotectant ramp was paused and the temperatures of the cephalon enclosure and the chiller were set to -3°C. The ramp was restarted at 12:15 and stopped again at 12:45 when jugular effluent measured over 100% of concentration necessary to vitrify (CNV).



Appendix D: Refractometry data

Perfusion continued at this concentration for 30 minutes, terminating at 13:15.

Visual observation of the brain showed the left hemisphere had retracted a few millimeters while the right hemisphere was still pressing against the skull (i.e., no dehydration). The face was evenly tanned except for a streak on the right cheek. Scalp coloration was irregular.

After removing the carotid cannulae, Kim's cephalon was removed from the enclosure and transferred to a modified LR40 dewar for cooldown to cryogenic temperature.

COOLDOWN

After placing Kim's cephalon in the modified LR40 dewar, installing the top, and connecting the temperature probes, cryogenic cooldown was started at 13:40 on January 17, 2013. When a temperature of -110°C was reached the cooling rate was reduced to minimize fracturing due to thermal stress. Once temperatures neared -196°C (approximately 96 hours after initiation of cooldown), the level of liquid nitrogen in the dewar was increased over a period of several hours until the cephalon was immersed. After reaching liquid nitrogen temperature, the cephalon was transferred to a Bigfoot dewar for long term care.

CT SCAN

Recently, Alcor has obtained CT scans of the heads and brains of neuro patients after cryoprotective perfusion. Originally performed as a means of determining the location and placement of acoustic "crackphone" sensors, it quickly became clear that much more than the crackphone sensors could be visualized. Using open source software to view CT scans, Alcor then developed color lookup tables (CLUTs) for various materials within the images in order to differentiate among things like bone, blood, ice, and cryoprotectant. The resulting images provide a long sought-after detailed view inside Kim's skull to help determine the efficacy of cryoprotective perfusion.

In general, patients who receive good care (i.e., immediate pronouncement of death, prompt stabilization, and short transport time) are well cryoprotected, while patients who experience problems suffer from some degree of perfusion impairment. The primary means of assessing the success or failure of brain cryoprotection until now has been to measure the retraction of the brain from the skull—a sign indicating dehydration of the brain as a result of successful uniform cryoprotective perfusion. With CT images, Alcor is now able to distinctly visualize such brains in three dimensions and to

assess the degree of perfusion impairment by inspecting the brain at various levels for evidence of areas of residual blood vs. areas of cryoprotectant.

Although a well-cryoprotected, dehydrated patient's brain is quite easy to distinguish, patients with varying degrees of perfusion impairment are more difficult to assess. A limited number of patient scans across various case conditions as well as a lack of experience in analyzing scans results in making a variety of assumptions regarding those cases. This is inevitable until more data can be gathered by performing more CT scans and then making comparisons across conditions.

In this case, Kim's medical power of attorney desired Alcor to use this new CT scanning technology in an effort to gauge the success of cryoprotective perfusion of the brain. Again, due to the limited number of scans performed so far, it is difficult to state observations with absolute certainty. However, several items are worth noting.

First, and in complete agreement with measurements of brain retraction during perfusion, there appears to have been very little dehydration of the brain overall. The left hemisphere is minimally dehydrated, while the right hemisphere shows no evidence of dehydration. This supports the view that cryoprotective perfusion was not generally successful.

Further evidence of poor perfusion can be observed when viewing images of Kim's brain serially using various CLUTs to differentiate areas of presumed impairment vs. cryoprotected areas. When viewed serially ~~there appear to be some areas of cortical cryoprotection, though minimal.*~~ The entire subcortex appears impaired.

Additionally, some anatomical abnormalities are distinguishable, such as the surgical site and entire areas of disturbance that are difficult to interpret but may, presumably, be caused by compression due to the presence of a space-occupying lesion (i.e., tumor).

Two factors have been hypothesized to have played a role in the poor cryoprotective perfusion of this patient's brain: (1) delay of pronouncement resulting in one hour of warm ischemia prior to commencement of stabilization procedures, and (2) high

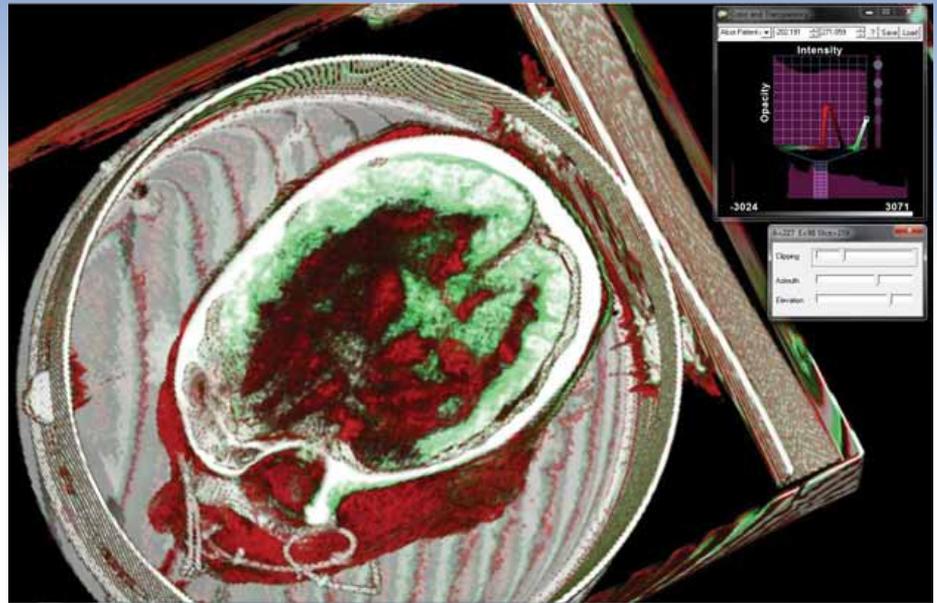
intracranial pressure caused by the tumor itself. While Kim certainly exhibited pre-mortem symptoms of increased intracranial pressure, such as headache and progressive loss of function (e.g., walking, speech, and ability to swallow), it is worth noting that the pattern of perfusion impairment observed in CT scans is entirely consistent with results from laboratory experiments in (non-pathological) rat brains undergoing similar periods of warm ischemia. It should also be noted that high intracranial pressure can also be a consequence of prolonged periods of warm and cold ischemia and resulting brain edema.

It would be quite useful and instructive to perform CT scans of several patients with space occupying lesions who were perfused under different conditions (e.g., immediate stabilization vs. delayed stabilization, short vs. long transport) to further elucidate the role of intracranial pressure in cryoprotective perfusion of the brain.

DISCUSSION

The primary reason for writing case reports and evaluating case performance is to determine whether, and how well, the objectives of stabilization and cryopreservation were achieved in a particular case (and, comparing cases over time, whether or not continuous improvements are made in meeting those objectives). Evaluations should be carried out while always keeping in mind the limitations under which a cryonics organization must operate in any given scenario. Such constraints will necessarily vary according to the particular details surrounding a given case. For example, all other conditions being the same, a patient who is pronounced close to a cryonics facility will benefit from a shorter (cold) ischemic period, which in turn may facilitate better cryoprotectant perfusion. For a fair appraisal, the most important question to ask is what could have *reasonably* been expected from a cryonics organization taking into consideration the constraints of the case.

In this case Alcor was informed well in advance of this member's condition and she was admitted to hospice quickly upon



Appendix E: Representative CT images

Figure A. A horizontal "slice" of a CT image at the level of the eyeballs. Using the CLUT for this image, we presume that red indicates unperfused tissue (i.e., electron densities similar to blood), while lighter colors (greens and whites) indicate cryoprotected tissue (i.e., electron densities similar to cryoprotectants). Under these assumptions, it appears that cortical tissue suffered the least impairment while subcortex was more severely impaired.

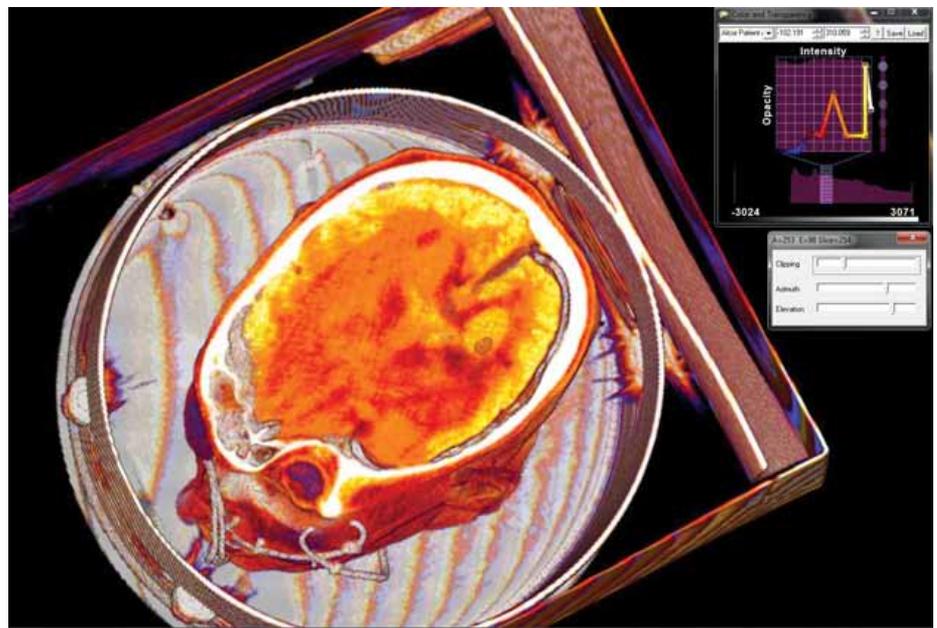


Figure B. A horizontal "slice" of a CT image at the level of the eyeballs (at the same level as Figure A). Using the CLUT for this image, we presume that darker colors (orange and red) indicate unperfused tissue (i.e., electron densities similar to blood), while lighter colors (yellows and whites) indicate cryoprotected tissue (i.e., electron densities similar to cryoprotectants). Again, we see the same pattern of perfusion impairment of the subcortex with uneven cryoprotection of the cortex.

More CT scans at: <http://www.alcor.org/Library/html/CTscan2643.html>

her arrival in Scottsdale. It is unfortunate, then, that she was discharged only one day prior to her pronouncement. This is, by far, the event having the most negative impact on this case.

Though she was relocated to a condominium near Alcor and the hospice advised that a staff member could be onsite within 10 minutes of being called, Kim's death apparently occurred at shift change and this situation ultimately resulted in a one hour delay between death and legal pronouncement of death, which is required before Alcor can take possession of the body. At least three other nearby long-term care facilities had been considered by the family prior to their decision to move her to a nearby condo because it gave them greater control over the administration of Kim's pain medication. There had been a number of disagreements between the patient's family and the hospice prior to Kim's discharge that make the delay in pronouncement that much more unsavory an occurrence. Whether another hospice would have provided better service is a question whose answer cannot be known. Ultimately, this is an issue between the cryonics organization and the member or her representatives.

Once pronouncement was made, stabilization moved smoothly except for a few problems primarily concerning batteries. Specifically, the DuaLogR batteries were dead prior to the start of stabilization, requiring team members to search for and obtain replacement batteries during stabilization activities. Contrary to protocol, only one temperature logger was used during stabilization, which further aggravated the problems with the DuaLogR. Additionally, the Lucas stopped working during CPS, resulting in a five minute period of (less efficient) manual chest compressions while the batteries were replaced. Another item, not described in the case report but found in the preparation notes, was that the battery of the rescue vehicle (RV) was also found to be drained upon inspection the day prior to the case. This situation is easily remedied by adopting a protocol for regularly testing power in all battery-operated equipment and replacing batteries when necessary.

The use of longer-lasting batteries is recommended as well, but testing and maintenance of equipment according to an established schedule is paramount if situations like those experienced in this case are to be avoided.

Given the condition of Kim the day prior to cardiac arrest, it may also be asked whether medications should have been drawn earlier. In the end, due to the late arrival of a nurse for pronouncement, it did not make a difference. However, if there had been a prompt pronouncement of death, medications would not have been drawn and ready at that time. Medications can be drawn when a patient's decline becomes apparent and may be kept stable by refrigeration (if necessary) until they are required.

Absence of the SCCD ("squid") from the PIB during initial stabilization procedures was another oversight and was not corrected until Kim had arrived at Alcor's facility (see Appendix A: Case Timeline), and even then not until a team member had asked about it several times. The use of a checklist for RV preparation would have easily identified any missing equipment and/or supplies, and is quick and easy to implement.

While there was an adequate number of team members to perform the most fundamental interventions (cardiopulmonary support, induction of hypothermia, and medications administration) for this case, and intervention was rapid once pronouncement was made, the only stabilization data collected was patient nasopharyngeal temperature. Additional data, including additional temperature collection, capnography and pulse oximetry (using the CO2SMO), and blood-gas analysis (using the i-STAT) could have been carried out given the number of team members available. This would have provided valuable information about how Kim responded to interventions.

Lack of data collection in this case was further aggravated by the absence of formal stabilization data collection sheets. Good data collection sheets not only improve the quantity and quality of data collected during a case, but also serve as a checklist to assist deployment of equipment prior to

legal pronouncement and to alert the scribe to errors and omissions during a case. Notes in this case were a conglomeration of time-stamped, free-form observations. Thankfully, due to the experience of the scribe during stabilization, they were comprehensive. But this became a bigger problem during surgery and cryoprotective perfusion, resulting in an incomplete description of OR events within the case report.

On the positive side, the fundamental interventions were carried out in parallel rather than in sequence, resulting in a very quick transport to the Alcor facility. Multiple IV access points were established, allowing medications administration to progress quickly. Additionally, an experienced team member served as scribe during stabilization, providing the team with a global view of the case as it progressed as well as a detailed set of notes for later case debriefing and reporting.

In the OR, surgery to isolate the carotid arteries was initiated with Kim still in the PIB and receiving chest compressions. This reduced the risk of patient warming during surgery and allowed for quick cannulation after cephalic isolation and transfer of the cephalon to the cephalon enclosure. Unfortunately, there was some confusion and disagreement regarding the ideal temperature at which to perform surgery. This has since been clarified, the fixed target temperature replaced by a target cooling rate. The new guidelines have been printed and placed in the procedures manual in the OR.

While surgery and perfusion were accomplished without incident, ~~the actual success of perfusion in this case appears negligible.*~~ A lack of brain dehydration, as seen in all patients under non-ideal circumstances of death and/or stabilization and transport, suggests that perfusion was significantly impaired. Further evidence from CT scans corroborates this assumption, revealing what is assumed to be significant perfusion impairment, minimal dehydration, and areas of significant pathology.

Two other basic procedures and observations that could provide more insight about the degree of ischemia /

edema, quality of cryoprotectant perfusion, and ice formation include the weighing of the cephalon prior and after cryoprotectant perfusion and inspection of the surface of Kim's brain between completion of cryogenic cool down and transfer to the Bigfoot dewar.

In general, a substantial gap remains between the amount of data that can be collected during a case and what is actually collected, reported, and analyzed. ■

* See Corrigendum for important updates to this article at:
<http://www.alcor.org/Library/html/CorrigendumA2643.html>

DETAILED TIMELINE

January 17, 2013

05:55	Respiratory arrest	07:20	Lucas stopped working, manual chest compressions begun	09:28	Nasopharyngeal temperature 18.4°C
06:10	Arrival of first stabilization team member	07:25	Lucas restarted (battery replaced)	09:33	Patient moved to OR table
06:25	Arrival of second stabilization team member	07:25	Temperature data logger started (batteries replaced)	09:43	Cephalic isolation
06:45	Arrival of third and fourth stabilization team members	07:28	Move PIB out of condo	09:45	Cephalon placed in holding ring of cephalic enclosure
06:46	Medications drawn while waiting for hospice nurse	07:30	Load PIB onto transport vehicle	09:51	Cephalon fell out of holding ring
06:59	Arrival of hospice nurse	07:45	Patient arrives at Alcor	09:52	Cephalon repositioned
07:00	Patient pronounced	07:49	Nasopharyngeal temperature 30.0°C	09:55	Carotids cannulated
07:02	Patient placed in portable ice bath (PIB)	07:51	Medications administration complete	09:58	Right carotid flushed
07:03	Start of mechanical chest compressions (Lucas)	07:54	Patient brought into operating room	10:00	Left carotid flushed
07:04	Left tibial IV line placed	08:00	Nasopharyngeal temperature 28.1°C	10:00	Nasopharyngeal temperature 17.2°C. Arterial temperature 5.3°C. Left venous temperature 23.7°C.
07:05	Additional ice added to PIB	08:02	Hair removal begun	10:01	Start washout (open circuit)
07:06	Administration of propofol	08:10	SCCD applied	10:19	Start cryoprotectant ramp
07:06	Administration of streptokinase	08:15	Hair removal completed	10:20	Start of closed circuit perfusion
07:07	Right tibial IV line placed	08:17	Incisions made for burr holes	10:38	Could not find right jugular vein
07:09	Bolus epinephrine administered, followed by slow infusion	08:22	Burr holes completed	10:38	Vascular resistance dropping
07:09	Administration of VitalOxy	08:31	Nasopharyngeal temperature 23.2°C	10:57	Observe eyeball shrinkage
07:10	Temperature data logger not working	08:36	Crack phone probes inserted in burr holes	11:16	Observe evenly tanned face, eyeball shrinkage, but little brain retraction
07:11	Nasopharyngeal thermocouple secured in left nostril	08:58	Ambu bag removed from King airway and more ice added to the PIB	11:20	Repositioned enclosure thermocouple, which was touching side of enclosure
07:13	Placement of King airway	09:00	Nasopharyngeal temperature 20.1°C	11:41	Ramp paused; enclosure and chiller temperatures set at -3°C
07:14	Administration of mannitol	09:05	Carotid isolation surgery started	12:15	Ramp restarted
07:15	Start of ventilation (Ambu bag valve mask, room air)	09:17	Left carotid isolated	13:15	End of cryoprotective perfusion
07:17	Nasopharyngeal temperature 31.4°C	09:20	Right carotid isolated	13:40	Start of cooldown