OPEN

Rapid Autopsy Programs and Research Support: The Pre– and Post–COVID-19 Environments

Jody E. Hooper, MD

Abstract: Each rapid autopsy is a powerful opportunity to supply multiple researchers with many valuable tissue specimens at the same time. Since the beginning of the development of rapid autopsy, the overriding organizing principle for all rapid autopsy programs has been that the samples or organs must be removed and processed as rapidly as possible. To accomplish this, some rapid autopsy programs are focused on only 1 tumor type, whereas others accept patients demonstrating all tumor types and sometimes other diseases as well. Rapid autopsy programs are logistically complicated and labor-intensive structures; therefore, the key to their success is program flexibility and maintaining a multidisciplinary focus. The necessary collaborations in the complex relationships between clinicians and researchers can be broken down into a series of thought and action steps that must be understood, accepted, and practiced by all participants. A crucial part of the precase steps (prior to death) for a rapid autopsy is the study consenting process. It is extremely important that this individualized consent is obtained for postmortem specimens and that it is written in terms general enough to be used for patients with all types of diseases and for an appropriate range of future research uses. The advent of SARS-CoV-2/COVID-19 (severe acute respiratory syndrome coronavirus 2/coronavirus disease 2019) has presented new challenges and opportunities to the field of autopsy pathology. Guidelines and practice had to be created and adapted to protect physicians and staff while maximizing diagnostic yield. However, any autopsy performed on a patient dying of or with COVID-19 represents a unique opportunity to contribute to understanding the disease mechanisms and to improve death certification, thus assisting in both clinical care and the development of health public policy.

Key Words: autopsy, cancer research, COVID-19, postmortem, rapid autopsy

(*AJSP: Reviews & Reports* 2021;26: 100–107)

Rapid autopsy has been shown to be a powerful tool for advancing research at essentially no risk and with no distress to the contributing patient. As such, rapid autopsy has contributed substantially to the understanding of metastatic spread since its earliest days. Rapid autopsies are postmortem examinations performed on an urgent basis (measured in hours) after the death of the patient, and sampling tissues using a rapid autopsy from patients with advanced malignancies provides unique research opportunities. First, tumor tissues can be procured in large quantities from many separate body sites. Second, neoplastic tissues can be sampled after disease resistance has occurred, allowing for studies to be performed on tissues after the time point of aggressive local spread or metastasis. In these instances, biopsy/surgical tissue is often not available or is accessible in sufficient quantity. Finally, different and often unique tissue samples for multiple researchers/research projects can be collected during a single case. Collection in short time intervals after the death of the patient means that rapid autopsy tissue quality can, in many instances, be considered comparable to fresh surgical biopsy tissue.[1]

RAPID AUTOPSY'S ROLE IN SUPPORTING RESEARCH

Table 1 gives current rapid autopsy programs (RAPs) within the United States, identified through internet and publication searches, as well as by telephone and other interactions with colleagues. As demonstrated in Table 1, hospitals/health systems currently supporting RA programs have several demographic characteristics in common: (1) a location in major metropolitan area with a large proximate population, (2) recognition as a distinguished cancer center (recognized by the US News & World Report) with many oncologists and oncology researchers, and (3) existence of a resource-rich environment with infrastructure for cancer research activity and experience with obtaining grant funding. These very characteristics imply potentially significant barriers of entry for possible new programs. A large nearby population is necessary for there to be a sufficient source of patients close enough to the center to facilitate transportation within a reasonable number of postmortem hours. Recognition as a distinguished cancer center seems necessary to have a sufficient audience of researchers willing and able to put the rapid autopsy tissue collected to immediate productive use. Experience and success in obtaining grant funding are important because most successful RAPs are or are moving toward becoming "self-sustaining" through grant funding. In addition to all of these characteristics, a RAP is a logistically complex and labor-intensive structure in which a dedicated and knowledgeable "champion" or founder with sufficient allocated time is a key to success.[2]

Examples of RAP Contributions to Cancer Research

Tissues harvested during rapid autopsies have successfully been utilized for DNA sequencing, RNA expression analysis (including in situ hybridization), proteomic approaches, and immunohistochemical studies in the field of prostate,[3–6] pancreas,[7] breast cancer,[8] and brain tumors.[9] In addition, these tissues have provided the opportunity to develop patient-derived animal models recapitulating the end stage of metastatic disease and to utilize molecular studies that have become milestones in understanding intratumor heterogeneity.[4,10]

A selective overview of major scientific contributions enabled by rapid autopsies includes activity by 3 of the longest-running RAPs in United States (ie, University of Michigan, Ann Arbor; University of Washington, Seattle; and The Johns Hopkins University, Baltimore) and offers some helpful insight into the research advances achieved so far. In 1996, the University of Michigan developed a RAP specifically for prostate cancer.[4] By 2003, the

From the Department of Pathology, The Johns Hopkins University, Baltimore, MD.
Reprints: Jody E. Hooper, MD, Department of Pathology, The Johns Hopkins University, 600 N Wolfe St, Baltimore, MD 21287.
E-mail: jhooper9@jhmi.edu.
This research was supported by an NIH Cancer Clinical Core Support grant (P30CA006973) and a supplement to an NCI Prostate SPORE grant (P50CA58236).
The author has no funding or conflicts to declare.

ISSN: 2381-5949
DOI: 10.1097/PCR.0000000000000435

TABLE 1. Overview of Nonneurologic Rapid Autopsy Programs in the United States

Location (City/State)	Metropolitan Area Population (Approximate)	Type/Size of Hospital (Beds)	Year Program Began	Program Administered by	Primary Focus	Recognition (See Legend Below)
Tucson, AZ	4,700,000	Private Health System	1987	Organizational administrator	All tumor and major tissue types, normal or diseased	n/a
Baltimore, MD	2,800,000	University/1177	2000	Pathologist	2000 Prostate, pancreas, breast 2014 All tumor types	1/2
Boston, MA	4,700,000	University/777	2016	Pathologist	All tumor types	1/2
New York, NY	20,300,000	University/473	2015	Pathologist	All tumor types	2
Ann Arbor, MI	5,300,000	University/998	1996	Medical oncologist/ pathologist	All Tumor type	1/2
Omaha, NE	975,000	University/809	2002	Researcher	Pancreas, prostate, other tumor types	2
Bethesda, MD; Washington, DC	6,200,000	National Clinical Research Center/ 200	2013	Researcher	Lung, thymic tumors, mesothelioma	n/a
Chapel Hill and Durham, NC	2,000,000	University/803	n/a	Pathologist	Breast	3
Columbus, OH	2,100,000	University/1506	2013	Oncologist	All tumor types	2
Pittsburgh, PA	2,400,000	University/770	2003	Oncologist	Lung, heart, vertebral shavings	2
Philadelphia, PA	7,100,000	University/695	2018	Obstetrician/ gynecologist	Ovarian	1/2
Seattle, WA	3,500,000	University/442	1989	Scientist/pathologist	Prostate	2
New York, NY	20,300,000	University/2410	2013	Pathologist	All tumor types	2

1 = US News & World Report 2020–2021 Best Hospitals Honor Roll (Top 20).

2 = US News & World Report 2020–2021 Best Hospital Rankings by Specialty—Cancer (Top 50).

3 = US News & World Report 2020–2021 Best Hospital Rankings by Specialty—Cancer (Higher Performance).

Adapted with permission from John Wiley and Sons.[2]

program had performed 30 rapid autopsies on patients with hormone-refractory prostate cancer. Researchers combined tissue microarray immunohistochemical analyses with hierarchical clustering of the cDNA expression pattern on many different metastases, including multiple bony sites for each patient. From this, they were able to show that metastatic hormone-refractory prostate cancer is characterized by heterogeneous morphology, immunophenotype, and genotype, reinforcing the concept that metastatic prostate cancer is a group of diseases even within the same patient and that obtaining metastatic tissue is of crucial importance to understanding the biology of cancer.[3] Later, the same group performed a groundbreaking study, using tissues and cell lines/xenografts obtained from their RAP, in which a highly recurrent gene fusion event was discovered to be a driver rearrangement in prostate cancer.[11]

Researchers at the University of Washington have also been performing rapid autopsies for prostate cancer for a number of years and have developed more than 30 patient-derived xenografts that have been widely distributed and used in many studies.[12] They have uncovered novel pathogenic mechanisms of prostate cancer progression, including a recent study showing that SRRM4 expression and the loss of REST activity may promote the emergence of the highly aggressive neuroendocrine phenotype in castration-resistant prostate cancer.[13]

At The Johns Hopkins University, rapid autopsies for collection of research tissue have been performed since the early 2000s, first concentrating on prostate, breast, and pancreatic cancers, and more recently expanding to all tumor types. In a 2010 study using rapid autopsies on patients with late-stage pancreatic cancers, the Hopkins group demonstrated that 30% of patients died with locally aggressive disease, rather than distant metastases, and showed a correlation between DPC4 genetic status by immunohistochemical staining at initial diagnosis and later extensive metastatic disease.[7] A separate landmark rapid autopsy study on patients with pancreatic ductal adenocarcinoma used mapping of the parental clone and subclones (identified by comparative lesion DNA sequencing within serial sections of the infiltrating pancreatic carcinoma) to show for the first time that primary pancreatic cancers contain a mix of geographically distinct subclones, each containing large numbers of cells that are present within the primary tumor and that may be detected years before the metastases.[14] This work revealed a quantitative "molecular clock" based on mutations; for example, the model revealed an approximately 15-year time span between creation of the initial founder cell and the development of metastases. This result has major implications for development of screening for early pancreatic cancer, increasing the likelihood of successful treatment in this highly lethal disease.[14]

From initially being the preserve of a few academic institutions, RAPs have recently been spreading throughout the United States, Canada, Australia, and very recently in Europe as well. Some of these programs are focused on only 1 tumor type, whereas others accept patients demonstrating all tumor types and sometimes other diseases as well.

A Sample Rapid Autopsy From Consent to Conclusion

A review of the sequence of events and sampling procedures for an example rapid autopsy case is helpful to illustrate factors

affecting case logistics, the importance of clear communication with research teams, and the unparalleled sampling opportunities afforded by autopsy. The limiting variable to research sampling at autopsy is not the amount of tissue available as has often been the case in other approaches to tissue sampling, but rather only the time and expertise of a skilled autopsy team. Prioritization of locations and types of specimens is key, as is an ability to customize sampling in real time during the autopsy as discoveries are made of treatment effects and new metastatic sites.

Background

The patient was a 71-year-old man with an 11-year history of prostate cancer. He was initially diagnosed when he was found to have an elevated prostate-specific antigen level and underwent a prostate biopsy that showed Gleason grade 4 + 5 = 9 prostatic adenocarcinoma. He then underwent a radical prostatectomy, which confirmed the Gleason grade, with a stage T3b N0 and negative surgical margins. Because of a rise in his prostate-specific antigen following surgery, he was treated with hormonal therapy and radiation therapy. He subsequently developed resistance to hormonal therapy and underwent multiple additional lines of treatment, including TAK-700, sipuleucel-T, enzalutamide, abiraterone with prednisone, and radium-223. He was then found to have bone metastasis involving his ribs, vertebrae, and pelvis. Treatment was started with nivolumab, docetaxel, and cabazitaxel. Despite the treatment, metastases progressed involving the liver and skull base, and the patient began to experience significant neurological symptoms attributed to his skull metastases. A rapid research autopsy was consented by the patient's legal next of kin, and less than a month after consenting, he died while in hospice care. After confirmation of the consent, the patient was transported to the hospital, and the autopsy was begun 5 hours after death. The rapid autopsy was attended by the lead pathologist, an autopsy assistant (diener), a specimen coordinator, a pathology postdoctoral fellow, 3 research associates from the prostate cancer research team, and a dedicated pathology photographer.

Tissue Sampling Procedure

The lead pathologist sampled all readily accessible metastatic sites with sterile scalpel and forceps, using fresh instruments for each site. Normal tissue controls were sampled in situ by the same method. Samples were placed on the dedicated cart covered by a sterile surgical drape and dissected by the postdoctoral fellow. Tissue sampling avoided macroscopically evident necrotic areas. Metastatic tissues were distributed to the prostate cancer team research associates. Fresh samples for cell culture were taken first with corresponding specimens snap frozen in liquid nitrogen and fixed in 10% neutral-buffered formalin for routine histopathologic processing. The specimen coordinator recorded all sample locations, dates and times of collection on specimen tubes, and a specimen manifest. All of the organs were subsequently removed from the body en bloc, weighed, and sectioned at every 1 to 2 cm. A hollow point drill was used to sample bone cores from vertebrae, including T10 to T12 and L1 through L5, as well as bilateral iliac crests. The adipose tissue adjacent to the greater curvature of the stomach had nodular areas, tan-white on cut surface. There were adhesions between the serosal surface of the duodenum and the surrounding soft tissue. The liver parenchyma had multiple firm tan-white nodules at the hilum and throughout the parenchyma, ranging from 0.2 to 3.5 cm in greatest dimension. The extrahepatic biliary system and portal vein were surrounded by adhesions and white-tan nodules. The pancreas had a nodular surface with a firm parenchyma. A firm tan-white nodule was found in the left adrenal gland. The left fourth and fifth ribs showed tan firm nodules on the interior surface, ranging from 0.1 to 0.2 cm in greatest dimension. Finally, there were firm tan-white nodules on the anterior surface of the thoracic and lumbar vertebrae, measuring up to 0.9 cm in greatest dimension. All the other organs, including brain and spinal cord were unremarkable.

Summary of an Example Rapid Autopsy Case

In summary, 27 metastatic sites (liver, pancreas, right and left adrenal, prostate bed, paraesophageal, perigastric, periaortic and mesenteric lymph nodes, sternum, right posterior chest wall, left fifth rib, T10–T12, L1, L3–L5, right and left Iliac crest) and 9 normal tissues (skin, skeletal muscle, heart, lung, pancreas tail, thyroid, kidney, liver, spleen) were sampled both with frozen tissue and formalin-fixed and paraffin-embedded tissue. A total of 76 specimens were harvested, including 4 fresh, 35 frozen, and 37 formalin-fixed. The prostate cancer team research associates also collected more than 100 specimens.

Postcase Activities

Postcase activities usually include the review of all histologic sections of metastatic samples and normal control tissues and recording the percentage of neoplastic tissue, viable tumor, and other cells. For specific cases, PowerPoint slides are prepared that summarized the clinical history of the patient, the rapid autopsy findings with photomicrographs, the analysis of tissue viability and the timing of tissue sampling. This material can then be circulated among the members of both the autopsy and research teams. One month after the autopsy, a joint meeting is held by the 2 teams to review rapid autopsy findings, make clinical-pathologic correlations, and discuss planning for follow-up genetic and other studies utilizing the samples, as well as goals for the upcoming cases.

Summary on RAPs as a Partner in Research

In the era of personalized medicine and as understanding of intercancer and intratumoral heterogeneity grows, rapid autopsies have been shown to be a powerful tool for advancing research at essentially no risk and with no distress to the contributing patient. Indeed, families of donors have very often testified that their experience in this type of postmortem donation is a rewarding way for them to contribute to science.[15] Academic and health centers with a substantial investment in cancer research should consider developing and using this emerging research support tool and evaluate to what extent these programs can contribute to their cancer research mission. As rapid autopsy becomes more widely accepted and utilized, it will be essential for those involved in these programs to communicate and share techniques and resources. It is hoped that over time this new research-oriented 21st-century purpose for the ancient field of autopsy will increasingly contribute to leading edge research in cancer and generally support research throughout the United States and internationally.

RAPID AUTOPSY PROGRAM ORGANIZATION AND LOGISTICS

Rapid autopsy programs are logistically complicated and labor-intensive structures; therefore, the key to success is program flexibility and maintaining a multidisciplinary focus. Flexibility is important to creating the appropriate team for a specific program environment and set of research goals. Many programs are on-call 24/7 often with at least 2 rotating teams that cover 1 or multiple week shifts. However, successful programs may also be run by only 1 team that is on-call for a restricted number of hours every day. These programs are usually led either by pathologists or oncologists, although integrated work between the 2 subspecialties

and specifically between clinicians and researchers is always crucial for the programs to thrive in the long term.[16]

Active Steps for Implementing a RAP

Successful collaborations in the complex relationships between clinicians and researchers can be broken down into a series of action steps given in Figure 1. The concept of utilizing postmortem tissue and the potentially available quantities of tissue is sufficiently new to some researchers that some careful a priori thought is required about how the RAP will fit into existing projects or create new avenues for investigation (CONSIDER). The patient population and sample types must be determined. Postmortem intervals (PMIs) can markedly affect how tissue may be successfully utilized, and parameters for different case scenarios should be delineated (DEFINE, MEET). The RAP must then be reviewed with potential participants and families (DISCUSS), and proper legal documents acquired (CONSENT). Pathologists and researchers and/or other scientists who perform the rapid autopsy must work closely prior to and at the time of collection (COOPERATE) and during evaluation and subsequent research (COLLABORATE). Lastly, rapid autopsy pathologists should create a communication loop about research results, family experiences, and evolving case needs (FEEDBACK).

The Study Consent

A crucial part of the precase activities (prior to death) is the study consenting process, in which the patient or his/her legal next of kin signs and gives his/her consent to collect and use his/her tissues for future research. The study consenting process may include explicit permission for a wide spectrum of possible future uses (such as genetic sequencing, generation of cell lines, tissue banking, sharing of tissue with researchers at different institutions, taking images of cases, retrieval of slides and blocks from prior biopsies and resection specimens, and the collection of premortem blood specimens). Of course, the patient can decide to restrict his/her consent as they may prefer.

It is extremely important that this individualized consent is obtained for postmortem specimens and that it is written in terms general enough to be used for patients with all types of diseases. It appears likely that such individualized consent will soon be required for genetic testing and cell lines, even in deceased patients, as regulatory agencies become aware of the expanding potential of postmortem tissue. In addition to the necessary study consent, rapid autopsies require a consent for the autopsy procedure itself, in the same way as regular diagnostic autopsies.

Sampling Goals and Approaches

Each rapid autopsy is a powerful opportunity to supply multiple researchers with many valuable specimens at the same time. For example, a single rapid autopsy case can provide tumor tissue to in-house researchers, tissue to 3 other out-of-state institutions (including National Institutes of Health), and banked tissue for the program itself, as well as normal controls of brain, eye, pituitary, heart, pancreatic duct, and skeletal muscle for other research

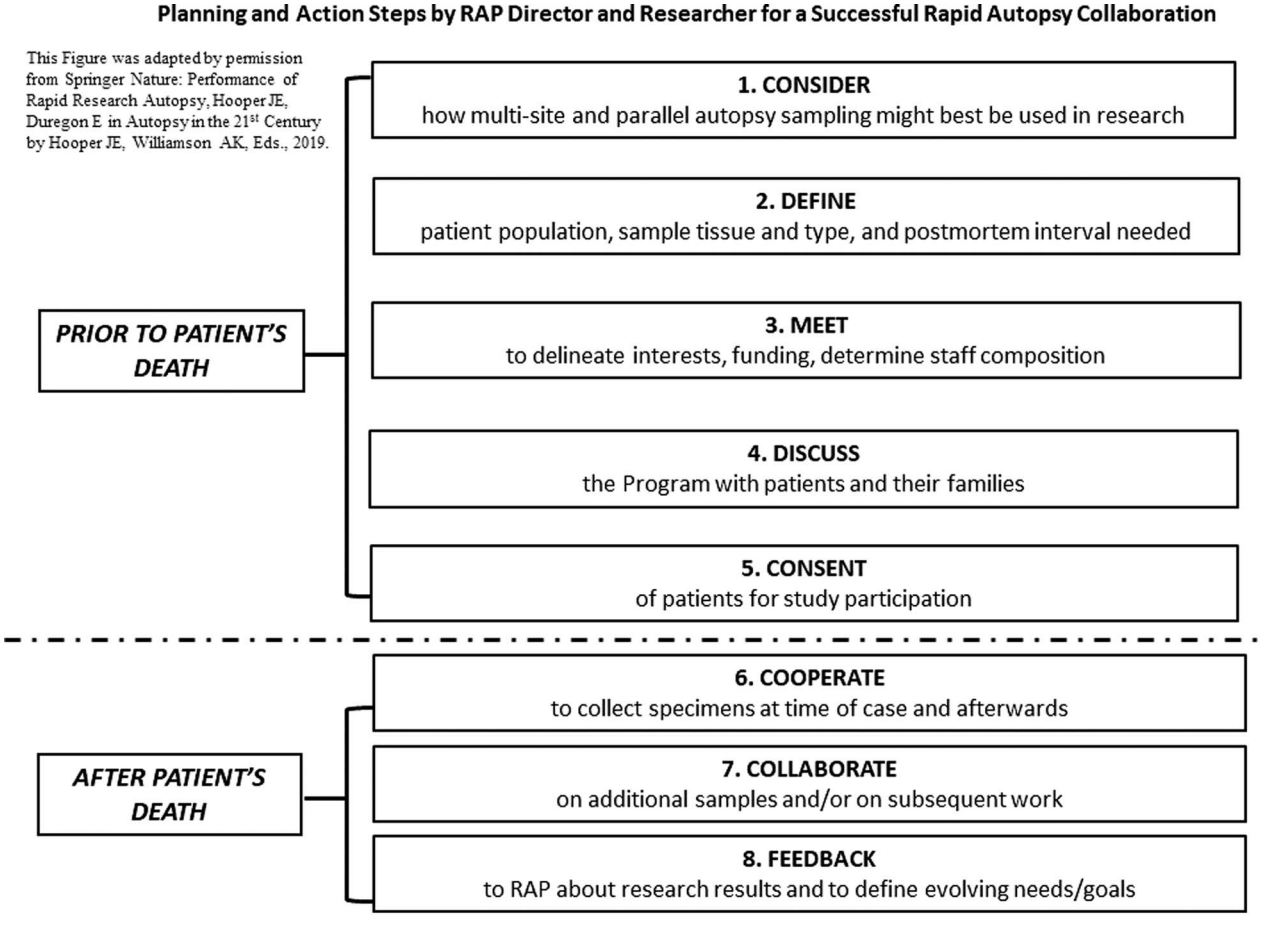

FIGURE 1. Planning and action steps by RAP director and researcher for a successful rapid autopsy collaboration.

groups. To accomplish this, it is imperative to have prearranged sampling protocols that specify types of tissue and processing necessary for this type of multifaceted action during a case. Each research group has a designated representative, and these people are contacted as a group as soon as the patient's death is known. Research teams provide supplemental personnel to help in the autopsy, with the extent and composition of these teams varying by the number of specimens sought and the extent of immediate on-site processing required. It can also be highly useful to sample each lesion/normal tissue in parallel by allocating part of the same area to be collected fresh in media such as RPMI, part flash frozen in liquid nitrogen or gradually frozen in OCT, and part formalin fixed. Corresponding studies utilizing cell lines or xenografts, sequencing, and immunohistochemical staining can create synergy, helping to delineate the "life cycle" of a cancer cell. It is important to have at least 1 team member dedicated exclusively to specimen labeling and tracking to facilitate this important parallel sampling.

Timing and Processing of Samples

Since the beginning of the development of rapid autopsy, the overriding organizing principle for all the RAPs has been that the samples or organs must be removed and processed as rapidly as possible. With this press for "high-quality" tissue comes the need to determine the critical markers of quality for human postmortem tissue.[17,18] The PMI is defined as the time elapsing between the time of death and the time the tissue has been placed in the preservative (either medium for fresh samples, snap freezing in liquid nitrogen, or fixation in formalin). Depending on the center and the particular aspects of the case involved, typical PMI can range between 0.5 and 23 hours. Postmortem tissue quality can be affected by PMI, premortem (agonal) conditions, and postmortem (preanalytical) factors. Traditionally, a low PMI has been the hallmark of high tissue quality.[19,20]

Although the effect of PMI on sampling can be highly individualized by type of cancer or disease and patient body habitus, some general PMI guidelines for successful sampling can be gleaned from the literature on the subject. As depicted in Figure 2, fresh samples with living cells are best gathered within 6 to 8 hours of death. Specimens for RNA and DNA sequencing may be frozen or placed in a media with stabilizing reagent and are generally best collected within 12 hours of the death at most. Histology and immunohistochemistry will still produce good results after 12 hours of PMI. However, the author has had the experience of cell lines growing from a sample taken after 12 hours when the setting (within an area of hemorrhage) was propitious. Rapid autopsy pathologists and researchers should discuss together what collections will be done if circumstances (family concerns, weather, traffic, or any of many other circumstances) might extend the PMI longer than had been planned.

Examples of Successful RAP Organizations

The varied approaches to creating and implementing the complex logistics of a RAP team will be highlighted in the 4 examples discussed below.

At the University of Nebraska Medical Center, a rotating research team is available 24 hours, 7 days a week, consisting of 2 full-time technicians, with participation of 3 on-call technicians and pathology assistants.[21] The RAP of the University of Michigan at Ann Arbor consists of 2 teams, an autopsy team and a tissue procurement team, which are alerted and work together during each case, and all are available 24/7. The autopsy team consists of a staff genitourinary pathologist, genitourinary pathology fellow, pathology resident, and a pathology assistant. The tissue

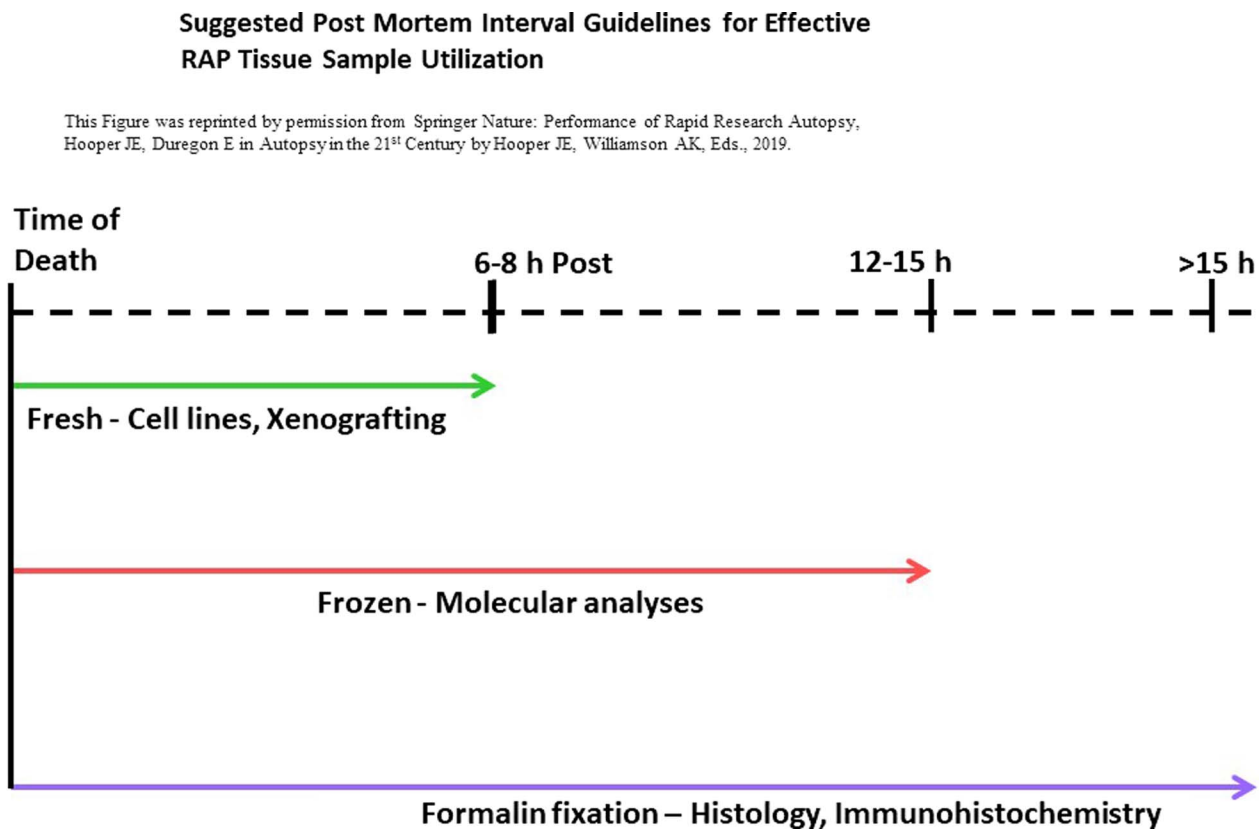

FIGURE 2. Suggested PMI guidelines for effective RAP tissue sample utilization.

procurement team consisted of the medical oncologist, staff and postdoctorate researchers, laboratory assistants, and a urology resident.[4] At Weill Cornell, the RAP team comprises an attending autopsy pathologist, a pathology resident, an autopsy technical assistant, and 2 to 4 additional members including pathologists, postdoctoral researchers, and research technicians who aid in tissue procurement and initial processing. The team may also include the patients' treating oncologists and surgeons.[22] The Johns Hopkins RAP team in Baltimore, Maryland, consists of a pathologist/RAP director, a specimen coordinator, an autopsy assistant/diener to assist in dissection, and a pathologist research autopsy fellow or instructor, and various cancer and other subspecialty research team members. The core team is available 7 days a week for 15 hours per day.

THE ROLE OF AUTOPSY DURING A GLOBAL PANDEMIC

The advent of SARS-CoV-2/COVID-19 (severe acute respiratory syndrome coronavirus 2/coronavirus disease 2019) has presented new challenges and opportunities to the field of autopsy pathology. Guidelines and practice had to be created and adapted to protect physicians and staff while maximizing diagnostic yield. At the same time, delineating a new disease has illuminated the unique power of multiorgan sampling that autopsy brings.

COVID-19 Autopsy Procedures

In March 2020, the US Occupational Health and Safety Administration issued guidelines that recommended against autopsy of COVID-positive patients.[23] Unfortunately, related to these guidelines, the contingencies of Decedent Affairs offices, and personal protective equipment shortages, multiple autopsy services including many at major institutions chose not to perform COVID autopsies. In some cases, performance of autopsies was shut down altogether. The US Occupational Health and Safety Administration guidelines were subsequently revoked, and the Centers for Disease Control and Prevention published recommendations for COVID autopsy performance. In a survey conducted by Dr Alex Williamson on an autopsy Listserv that began in May 2020, approximately 50% of surveyed participants were at institutions performing COVID-positive autopsies (A. K. Williamson, email, March 24, 2020). Most were performing autopsies that were modified in some way from the standard autopsy procedure.

Current Centers for Disease Control and Prevention recommendations and international guidelines suggest the addition of airborne precautions to standard and contact precautions including the use of an Airborne Infection Isolation Room with negative pressure and at least 6 changes of air per hour, or the best protective environment available.[24] Personal protective equipment should include at least an N95 mask or an equivalent personal respirator. The use of an oscillating saw is not suggested, although a vacuum shroud could be used to protect against aerosols. At our institution, we modified the autopsy procedure substantially. Our guidelines were arrived at in active discussion with our complete autopsy staff and trainees, taking into account not only their safety but also their emotional comfort levels with the procedure. COVID autopsies were performed in a separate negative-pressure suite, and all surfaces were thoroughly cleaned and bleached prior to removal of the patient in the closed body bag. Pre-COVID, we typically used a modified Letulle or en masse technique, removing all organs in 1 block and then dissecting. For COVID cases, however, we remove the chest organs for evaluation but perform in situ sampling or individual organ removal for abdominal and pelvic organs unless other specific investigation is warranted by questions in the clinical history. An oscillating saw is not used. All organs and tissues are fixed for 48 hours prior to sectioning.

Initially, no brains were removed from COVID autopsies, while a vacuum shroud for the oscillating saw was on order. However, an alternative method for brain removal arose as a result of a research project. Dr Matthew Stewart, an otolaryngology–head and neck surgeon, had attended COVID cases to sample mastoid and middle ear tissue, a project that ultimately demonstrated the

TABLE 2. Ongoing COVID-19 Autopsy Research at The Johns Hopkins University

Subject Area	Specialty	Organ(s)	Type of Sample	Status of Project
Presence of SARS-CoV-2 in middle ear	Otolaryngology—head and neck surgery	Mastoid, middle ear	Fresh for polymerase chain reaction	Published *JAMA Otolaryngology*, ongoing
Demographic, disease, pathologic features	Pathology	All sampled	Autopsy report data, fixed for histologic evaluation	Published, *Arch Pathol Lab Med*
Serum markers, complement cascade	Rheumatology	Lung, heart, kidney	Blood/serum, fixed for IHC	Submitted, under revision
Immune cells in response to infection, mechanisms of damage	Immunology/pathology	Heart, lung, kidney, liver	Frozen for flow cytometry, fixed for IHC	In progress
Endothelial damage	Cardiology	Heart, lung, kidney, liver, skin	Fixed for IHC	In progress
Development of IHC, in situ	Pathology	Heart, lung, kidney, liver	Fixed for IHC	In progress
Lung pathology	Pulmonology	Lung, bronchi	Fixed, other testing	In progress
Histology and ultrastructure	Pathology	Lung	Fixed for histology and EM	In progress
Presence and spread of virus	Neurology	Vagus nerve, skeletal muscle	Fixed for IHC	In progress
Effects on brain	Neurology, neuropathology	Brain	Fixed in methanol and formalin	In progress
Effects of SARS-CoV-2 infection	Otolaryngology	Trachea	Fixed	In progress

EM, electron microscopy; IHC, immunohistochemistry.

presence of viral DNA in these spaces.[25] In the process of this activity, he realized that the calvarium could also be removed using a similar technique with hand tools, and subsequently, brains have been removed in 10 cases to date, with portions of brain tissue sampled in 2 additional cases.

The Johns Hopkins Hospital autopsy service follows a COVID testing policy that mirrors that of the Department of Surgery for the most invasive and high-risk procedures. This includes a history screening with questions about symptoms, travel, and possible exposures. Outside patients with histories suggestive of COVID are not brought in for autopsy. Inpatients from within the hospital system must have had polymerase chain reaction testing with result within 5 days of the autopsy, and decedents coming from outside the hospital must have had testing within 2 days. If testing has not been performed within these time parameters, stat testing from nasopharyngeal swabs is performed, and the combined history and these test results dictate what autopsy procedure will be followed.

COVID-19 Research at The Johns Hopkins University

The RAP at The Johns Hopkins Hospital was suspended from March to October 2020, because of the necessity for personal protective equipment conservation and the requirement to develop a testing protocol for decedents being brought in from outside the Hopkins hospital. During that time, resources and techniques utilized for the RAP were redirected to COVID research. The same generalized research autopsy study consent was approved for COVID cases and signed by the next of kin at the time of consenting for autopsy. Researchers collaborated with the autopsy director to design projects. Fresh, slow-frozen, and formalin-fixed specimens, as well as samples in glutaraldehyde for electron microscopy, were collected for researchers in parallel with samples for clinical diagnosis. Flash freezing was not used because of the risks of aerosolization. Ongoing research investigations from these COVID cases are outlined in Table 2. It is important to note that postmortem specimens may be utilized in combination with blood and tissue samples that were taken during life, as is currently being done in studies of serum markers and the complement cascade. Multiple immunohistochemical studies, development of an in situ hybridization assay, and flow cytometry are all now being performed to evaluate the character of responding inflammatory cells. Autopsy samples are also being used as controls in the development of clinically used immunohistochemical stains as well.

However, COVID autopsies at Johns Hopkins have not been performed on extended hours, and work with fixed tissue would not necessarily require the type of individualized consent used for ethically sensitive work such as cell lines or genetic sequencing. This means that any medical center currently performing COVID-positive autopsies could participate in these types of research collections. Fixed samples could even be shipped to other centers, if requested.

In addition to the collection of specimens specifically for research, any autopsy performed on a patient dying of or with COVID-19 represents a unique opportunity to contribute to understanding of disease mechanisms and to improve death certification, thus assisting in both clinical care and the development of health public policy. Pathologists with the willingness and skill to perform these procedures, supported by the administration of their medical centers, will be able to demonstrate the true value of autopsy in the modern era.

INTO THE FUTURE

Rapid autopsy is a thoroughly modern application and contribution for an otherwise foundational medical technique and a chance for the practitioner of autopsy to demonstrate extraordinary value in a fresh new way. While rapid autopsy can supply tissue for developing new genomic and proteomic studies, it can and should also fulfill the worthy purposes that all autopsies can fulfill: diagnostic accuracy, education, training, and information and closure for family members and colleagues.

REFERENCES

1. Stan AD, Ghose S, Gao XM, et al. Human postmortem tissue: what quality markers matter? *Brain Res* 2006;1123:1–11.
2. Duregon E, Schneider J, DeMarzo AM, et al. Rapid research autopsy is a stealthy but growing contributor to cancer research. *Cancer* 2019;125(17): 2915–2919. doi:10.1002/cncr.32184.
3. Shah RB, Mehra R, Chinnaiyan AM, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. *Cancer Res* 2004;64:9209–9216.
4. Rubin MA, Putzi M, Mucci N, et al. Rapid ("warm") autopsy study for procurement of metastatic prostate cancer. *Clin Cancer Res* 2000;6: 1038–1045.
5. Grasso CS, Wu YM, Robinson DR, et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 2012;487:239–243.
6. Juric D, Castel P, Griffith M, et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kα inhibitor. *Nature* 2015;518:240–244.
7. Iacobuzio-Donahue CA, Fu B, Yachida S, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* 2009;27:1806–1813.
8. Avigdor BE, Cimino-Mathews A, DeMarzo AM, et al. Mutational profiles of breast cancer metastases from a rapid autopsy series reveal multiple evolutionary trajectories. *JCI Insight* 2017;2.
9. Kambhampati M, Perez JP, Yadavilli S, et al. A standardized autopsy procurement allows for the comprehensive study of DIPG biology. *Oncotarget* 2015;6:12740–12747.
10. Xie T, Musteanu M, Lopez-Casas PP, et al. Whole exome sequencing of rapid autopsy tumors and xenograft models reveals possible driver mutations underlying tumor progression. *PLoS One* 2015;10:e0142631.
11. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005;310: 644–648.
12. Roudier MP, True LD, Higano CS, et al. Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. *Hum Pathol* 2003;34: 646–653.
13. Zhang X, Coleman IM, Brown LG, et al. SRRM4 expression and the loss of REST activity may promote the emergence of the neuroendocrine phenotype in castration-resistant prostate cancer. *Clin Cancer Res* 2015;21: 4698–4708.
14. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010;467:1114–1117.
15. Alabran JL, Hooper JE, Hill M, et al. Overcoming autopsy barriers in pediatric cancer research. *Pediatr Blood Cancer* 2013;60:204–209.
16. Hooper JE, Duregon E. Performance of rapid research autopsy: best practices and future directions. In: Williamson AK, ed. *Autopsy in the 21st Century.* Cham: Springer Nature Switzerland; 2019.
17. Hargrove JL, Schmidt FH. The role of mRNA and protein stability in gene expression. *FASEB J* 1989;3(12):2360–2370.
18. Barton AJ, Pearson RC, Najlerahim A, et al. Pre- and postmortem influences on brain RNA. *J Neurochem* 1993;61(1):1–11.
19. Harrison PJ, Heath PR, Eastwood SL, et al. The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: selective mRNA vulnerability and comparison with their encoded proteins. *Neurosci Lett* 1995;200(3):151–154.

20. Lewis DA. The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology* 2002;26(2):143–154.

21. Ghorpade A, Bruch L, Persidsky Y, et al. Development of a rapid autopsy program for studies of brain immunity. *J Neuroimmunol* 2005;163(1–2): 135–144.

22. Pisapia DJ, Salvatore S, Pauli C, et al. Next-generation rapid autopsies enable tumor evolution tracking and generation of preclinical models. *JCO Precis Oncol* 2017;2017.

23. Paxton A. Autopsies show many faces of COVID-19. *CAP Today.* 2020.

24. Collection and submission of postmortem specimens from deceased persons with known or suspected COVID-19, interim guidance. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-postmortem-specimens.html. Updated November 2, 2020. Accessed November 12, 2020.

25. Frazier KM, Hooper JE, Mostafa HH, et al. SARS-CoV-2 virus isolated from the mastoid and middle ear: implications for COVID-19 precautions during ear surgery. *JAMA Otolaryngol Head Neck Surg* 2020;146(10):964–966.