GUEST EDITORIAL

The Clinical Autopsy and Genomic Testing

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Autopsies were crucial to the advancement of medical knowledge and the understanding of human disease well into the 20th Century. Today, clinical autopsies—autopsies performed on patients who die of natural causes—are conducted in hospitals mostly as a means of quality control for the accuracy, thoroughness, and effectiveness of diagnosis and treatment. However, improved methods of imaging, blood-based testing, and minimally invasive biopsies have increasingly led to the assumption by many clinicians that autopsies are of declining value for uncovering abnormalities unsuspected during life. In addition, the sizable costs of performing an autopsy are not reimbursed by a third party and must be borne solely by the hospital, except for those rare instances when the family pays. For these and other reasons, the Joint Commission for the Accreditation of Hospitals almost 50 years ago abandoned the requirement of a minimum number of autopsies for hospitals to retain certification. Nevertheless, many studies have concluded that properly performed autopsies still find disease that has been missed or misdiagnosed in life and continue to yield valuable information, at least in part because of the constantly increasing number of described disease entities, the need for the objective evaluation of efficacy for an ever-expanding range of therapies, and the risk of adverse effects that may be associated with those therapies.^{1–3}

Irrespective of their value for quality control, autopsies remain a formal aspect of the training in pathology residencies. Autopsies educate trainees in gross morphologic and histologic diagnosis, especially through extensive exposure to the wide variations that exist within normal human organs and tissues. Furthermore, integration of the postmortem findings and clinical history develops understanding on the part of the trainee in the pathogenesis of disease and the dynamics of disease progression. However, a carefully performed autopsy is a labor-intensive, time-consuming undertaking in an era when trainees are asked to assume a broad array of duties and are expected to assimilate an imposing amount of knowledge. The competition for the pathology trainee's time and attention, together with the waning interest in autopsies within the general medical

community, has worked to deemphasize the autopsy in the training of young pathologists in many institutions. This deemphasis is reflected by the lack of fellowships or board certification in clinical autopsy pathology; and in most academic centers, autopsy services are staffed by faculty pathologists whose primary interests lie elsewhere, often in research unrelated to autopsy pathology.

Taken together, all these factors have tended to diminish the role of the autopsy in discovery, education, and oversight of quality in clinical care. What if anything can or should be done to restore the status of the autopsy in modern medicine? Perhaps more important, is there a legitimate place for the autopsy as a driver of research, learning, and improved medical practice in the contemporary world? One answer to both these questions could be to include sequence analysis of the patient's genomic, germline DNA as a routine part of the clinical autopsy.

Next-generation sequencing was first introduced about 15 years ago as a technology by which the nucleotide sequencing of many regions of DNA can be analyzed simultaneously (so-called massively parallel sequencing). Subsequent methodologic and bioinformatic improvements have rendered sequencing of large regions of genomic DNA relatively straightforward and quick. Genomic DNA inherited through the germline remains largely intact within dead tissues for long periods of time and is easily extracted from any bodily tissue, even after formalin fixation and embedding in paraffin. Major efforts are currently underway to use DNA sequence information obtained from individual patients to diagnose diseases and identify targets for drugs that may counteract the effects of inherited (and in the case of cancer, acquired) mutations in specific genes. This paradigm constitutes the essence of what has been termed

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personalized or precision medicine—therapy tailored to the individual patient based on pathogenic variants in sequence found within the patient's DNA. Concurrently, analysis of inherited variants and their effect on disease or disease susceptibility has become an active field of preclinical medical research over the past decade and a half.

Screening of genomic DNA as a routine feature of the clinical autopsy would be entirely consistent with the widely practiced policy that autopsies unrestricted by the family to a particular organ or bodily region should be a comprehensive examination of the physical state of the deceased. All organs are examined grossly, and samples are collected randomly from each tissue for microscopy, in addition to any specific macroscopic lesions that might be found. Beyond the gross and microscopic examination of the organs in each autopsy, body fluids might be analyzed for biochemical abnormalities, blood and tissues cultured for organisms, and body parts X-rayed. Some of these procedures may be limited to cases with certain histories or gross lesions, but others, such as culture—a general screen for bacterial infection—are often routine for all autopsies. However, one type of diagnostic procedure rarely applied to the autopsy is genomic screening for disease-related inherited sequence variants in DNA.

Aside from genetic testing to detect specific pathogenic mutations for which there is strong evidence or suspicion based on clinical diagnoses, a variety of considerations now argue for inclusion of broad-based DNA sequencing as an integral part of the routine autopsy: i) Inherited mutations and DNA sequence polymorphisms sometimes account for or contribute to disorders that may otherwise appear sporadic, so that the true etiology of a disease may not be discernible without sequence analysis. ii) Inherited mutations and polymorphisms may be germane to disease or disease diatheses in relatives of the deceased. iii) Routine genomic screening of deceased individuals offers the opportunity to make new correlations between genotype and disease, including the chance to discover new genetic disorders. iv) Exposure of pathology trainees to genomic analysis and its interpretation would broaden the familiarity of trainees with the principles of genomics and bioinformatics that will be increasingly valuable in all fields of medicine moving forward. v) Viewed from the perspective of those who continue to appreciate autopsies as a vehicle for training, disease discovery, and the acquisition of tissues for research, genomic screening might provide a way to promote renewed interest in autopsies within the medical establishment and reinvigorate this traditional but still worthwhile procedure.

Besides the potential benefits of genomic screening as part of the routine autopsy, the possible implementation of DNA sequencing as a routine part of the autopsy raises several concerns. A prime example is cost. Actually, the steadily decreasing cost of next-generation sequencing, using either whole exome (the coding sequence of all roughly 20,000 human genes) or less expensive subsets of genes known to be associated with inherited disorders, would contribute a

relatively small increment to the total cost of autopsies. The cost of a clinical autopsy in the United States now averages approximately \$2000 to \$4000; the cost of whole exome sequencing is currently as low as \$300 to \$400. When the whole exome or large panels of genes are sequenced, bioinformatic costs may be reduced by prioritizing the analysis of selected genes in light of the clinical history and autopsy findings, and the data on other genes may be stored for later use. This approach, however, has the disadvantage of decreasing the chance of discovering new genetic disorders. (Whole genome sequencing is also possible but probably not feasible until the costs for sequencing and data processing undergo further reduction. Sequencing of mitochondrial DNA is not ordinarily performed as part of whole exome sequencing and presents certain special issues but might also warrant inclusion in the genomic analysis of at least some patients.)

In addition to the costs of sequence analysis and the part of bioinformatic processing of raw data that is not automated, there would likely be ancillary costs, such as the increased demand on genetic counseling services by family members who will want an interpretation of the results. These costs will have to be calculated and covered. Similarly, findings generated by routine genomic screening during autopsies may prompt increased requests for follow up sequencing of relevant genes in living relatives, the charges for which would most likely fall upon patients or their insurers.

Other concerns relate to matters of permission for testing, confidentiality, and potential misuse of genetic information. As a routine part of the autopsy, genomic screening would not be research per se, but even if it were, research on tissues from diseased patients does not generally require approval of institutional review boards. A permit signed by the next of kin is required for each clinical autopsy, and it should be not be difficult to include in an institution's standard autopsy permit a clear and frank explanation of the process (including the fact that no special sampling of tissue is necessary), the possible benefits, and the complications that may come with genomic testing. A modified autopsy permit may also offer options for the next of kin to decline to be informed of the results of genomic analysis or to deny genomic analysis entirely. Comparable statements describing the process and attendant risks have been incorporated for several years into consent forms presented to living patients who undergo genomic testing for inherited disease or for testing of tumor DNA, which may also sometimes reveal pathogenic germline mutations. Suitably edited versions of these statements may serve as models for insertion into the autopsy permit template.

Confidentiality should be no more complicated than it is for any other autopsy finding, including those with genetic implication for relatives, such as Mendelian disorders like hemochromatosis, α -1 antitrypsin deficiency, neurofibromatosis I, inherited amyloidosis, and various cancer predisposition syndromes, as well as complex, multifactorial disorders with significant genetic components, such as accelerated atherosclerosis and neurodegenerative

diseases—all of which are regularly documented in current autopsy reports. Furthermore, relatives who sign autopsy permits often explicitly seek information that might affect their own health or that of their families. The routine autopsy performed today seldom produces answers pertinent to these questions and inclusion of genomic testing as a part of the autopsy would greatly help in providing informed responses to these queries.

Decisions will have to be made about who performs the sequencing analysis, who reports the findings, and what is reported. Sequencing might be accomplished by core facilities and clinical laboratories that have been established in many academic centers or could be obtained through commercial sequencing organizations. Analysis of primary data to identify clinically meaningful inherited sequence variants may or may not be part of the services offered by in-house sequencing facilities and requires skills in which most autopsy faculty or staff, let alone trainees, are not often presently experienced. However, increasing numbers of pathology departments now have laboratories that sequence tumor DNA for cancer precision medicine, and there is significant overlap between the interpretation of sequence data from tumors and from normal tissues. Furthermore, clinical geneticists in many institutions are well versed in the assessment of inherited sequence variants and may provide highly constructive advice in this area.

Pathologists may take the lead in reporting genetic findings from autopsies, but the responsibility for presenting the results to the families and discussing their ramifications probably best belongs with the genetics counselors, who possess the appropriate expertise for these functions. These functions should include the management of possible legal obligations to inform family members when the individual who signs the autopsy permit refuses to contact relatives who may be at risk for carrying a deleterious mutation.

Different autopsy programs may set different thresholds for reporting sequence variants on the basis of varying levels of evidence for pathogenicity and benignancy, or as modifiers of response to drugs, physical injury, disease, psychological stress, antigenic stimuli, or other environmental factors. The list of variants specifically cited in the report might be linked electronically to a fuller inventory of variants detected in the case. Which variants are communicated to the next of kin will call for further judgment that realistically weighs the potential impact of a variant on the future health and medical care of living relatives. Clinical geneticists and genetics counselors deal with similar situations on a regular basis, and detailed guidelines have been published on the topic.⁴

Several medical institutions have recently initiated investigative programs that screen the genomes of living patients to link genetic variants with diseases and conditions, ultimately to enable the prediction of future disease and allow preventive interventions in individual patients.^{5,6} Because the essence of the autopsy is a full description of the final physical state of the patient's body, accompanied by a close review of the accumulated, lifelong clinical

history, the ability to make such correlations would only be enhanced by using autopsies for this purpose rather than by relying exclusively on testing of living patients.

The prevalence of pathogenic variants expected to be identified by routine genetic screening in autopsies is dependent on several assumptions, but the rate at which such variants are detected may come close to that with which clinically significant diagnostic errors are now revealed by standard autopsies.¹ As recently reported, among a large cohort of >50,000 randomly selected volunteers in one integrated health care system, 3.5% were found to harbor loss-of-function mutations within a set of 76 clinically actionable genes.⁷ The rate for detecting clinically significant variants may be even higher in certain categories of autopsies. For instance, late-term stillbirths not infrequently pose diagnostic dilemmas about the cause of death, some of which might be due to inherited genetic variants without clear-cut anatomic manifestations. Autopsies of patients with an unusual set of unexplained findings or abnormalities that have not as yet been recognized as a genetic syndrome constitute another such group. Genomic testing may also have increased value in autopsies of patients with histories and signs of the unusually early onset of a chronic disease. In addition, as alluded to above, genomic sequence analysis may be particularly helpful in identifying germline mutations and rare polymorphisms that underlie some fraction of cases carrying particular diagnoses, such as cardiomyopathies and arrhythmias, diabetes and other metabolic disorders, developmental abnormalities, intellectual disabilities, and certain mental disorders, such as schizophrenia, to name just a few.

It should be recognized that definitive attribution of diseases and disorders to DNA sequence variants may be technically challenging. Pathogenic variants uncovered by genomic screening must be separated from neutral variants that have little or no effect on health or disease. Genomic screening as part of the routine autopsy will not ordinarily be aided by DNA analysis of pedigrees involving relatives displaying or lacking a similar phenotype alongside that of a proband. DNA analysis in pedigrees may be possible after obtaining results from the autopsy screen, but depending on the number of cases in which variants of uncertain significance turn up, the added expense and effort may make DNA analysis of pedigrees on a large scale impractical, unless reimbursed by insurance or some other outside source. However, several databases that catalog genetic variants associated with disease are publicly available [eg, Online Mendelian Inheritance in Man⁸ (an online catalog of human genes and genetic disorders), <https://www.omim.org>, last accessed May 6, 2019; and The Human Gene Mutation database, <http://www.hgmd.cf.ac.uk/ac/index.php>, last accessed May 6, 2019]. Other databases archive known DNA sequence polymorphisms along with their frequencies among various races and ethnicities (Single Nucleotide Polymorphism database, <https://www.ncbi.nlm.nih.gov/snp>, last accessed May 6, 2019). Also, algorithms accessible on the Internet predict the effect of genetic variants on the

structure and function of proteins.^{9,10} These programs can be useful in sorting physiologically important genetic changes from changes with little likely clinical significance. In any event, collecting and evaluating genetic data in light of a full diagnostic assessment of the deceased and a complete clinical history should help populate more extensive databases that will facilitate the future interpretation of results from the genomic screening of patients both living and dead.

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