The Utility and Scope of Forensic Histopathology

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ABSTRACT

Forensic histopathology is the use of histology to aid in the identification of disease and injuries in forensic pathology practice. The value of routine microscopy has been challenged in various studies and discussions have taken place in forensic journals about how useful microscopic diagnosis is in medicolegal autopsies. This paper reviews the literature on the value of histological examination in forensic practice and discusses routine histochemical stains that can be used in postmortem examinations to aid in the diagnosis and add value by confirming or refuting macroscopic findings. *Acad Forensic Pathol.* 2018 8(3): 426-451

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INTRODUCTION

The autopsy existed long before the advent of microscopy, with the use of the microscope to make pathologic diagnoses commencing in the 19th century. Today, histology is the core science for diagnosis in anatomical pathology (1, 2). Forensic pathology, however, remains an essentially naked eye discipline for many cases, supplemented with ancillary testing in appropriate cases, most commonly toxicology and histology. Forensic histopathology as a specific area of forensic pathology has developed, and has its own literature and textbooks (3). Certain areas are peculiar to forensic pathology and microscopic changes seen in certain conditions are only recorded in the forensic literature. This paper provides an introduction to the uses of histology in forensic pathology practice, examines its utility, and discusses typical types of stains and their value in the diagnosis of postmortem conditions. This paper does not discuss more specialized areas of autopsy practice in any detail, particularly neuropathology, which uses a large number of specialist histochemistry and immunohistochemical stains.

DISCUSSION

The Scope of Forensic Histopathology

Unlike surgical pathology, forensic pathology has a different purpose in trying to determine mechanism, cause, and manner of death and provide relevant information for the legal fora. While there is some overlap, there are certain conditions that are seen in medicolegal autopsy practice that are unlikely to be encountered in routine surgical pathology. Some main areas of histology in forensic pathology practice include the identification of specific disorders that require microscopy to identify them such as myocarditis, amniotic fluid embolism, and fat embolism. Certain pathologies are limited to autopsy histology such as hypothermia (**Image 1**), electrocution, drowning, and fire deaths (**Image 2**) which all have histological features that aid variably in their diagnosis (3). Injuries including bruising, lacerations, and fractures have their own microscopic appearances. Much time and energy has been spent on trying to time injuries histologically,

but with more limited application in real cases than is always anticipated. Systemic responses to injury, including pulmonary embolism, ischemia, and sepsis, can also be supported by microscopic examination.

Utility of Histopathology in Forensic Pathology Practice

There are certain investigations that by custom and practice have developed to require extensive histological examination. These include sudden deaths in infancy and maternal deaths. Such deaths, however, form a minority of most practicing forensic pathology casework and the value of microscopy in other investigations is open to more debate. Publications on the value of microscopy in medical (consent) autopsies have shown that there can be significant discrepancies between gross and microscopic diagnoses. Benardi and colleagues examined 1273 medical autopsies and found the largest discrepancies in the lungs 38.7%, liver 35.1%, and kidneys 30.3% (4). Hunt and colleagues also showed that discrepancies between macroscopic and microscopic diagnosis of bronchopneumonia were common (5). Roulson and colleagues did a meta-analysis of the literature on the difference between clinical and autopsy diagnosis and the value of postmortem histology. They concluded that over 20% of clinically unexpected autopsy findings, including 5% of major findings, could only be diagnosed histologically (6).

Studies have examined and reported the value of routine histopathology in forensic pathology practice. A 2006 study by Molina and colleagues from Bexar County, Texas concluded that routine histopathology in medicolegal cases was not necessary. They found that histology changed the cause of death in only one of 189 cases studied (a case of leukemia). The manner of death was not changed in any case (7). The selected cases were defined as when a cause and manner were evident by gross findings and in which microscopy was not normally conducted. The mean age was 41 years. Natural deaths made up 25% of cases; accidents were the largest group forming 36% of cases and homicides accounted for 27%. The most common causes of death were gunshot wounds (22%) and blunt force

injuries (22%). They stated that their findings and practice were in line with standards proposed by the College of American Pathologists and the National Association of Medical Examiners, which essentially allow professional discretion in whether histology is examined or not.

In another study published in 2006 from Sydney, Australia, Langlois analyzed 638 adult medicolegal autopsies for the value of histology (8). The age range was 16-98 years with a mean and median of 53 years. In this series, there were a significant number of histology blocks taken, with 3088 blocks of lung from 638 cases, 3392 blocks of left ventricle from 636 cases, and 1260 renal blocks from 636 cases, for example.

He noted that there was a high degree of discordance between macroscopic and microscopic diagnoses of the lungs, particularly in relation to the diagnosis of pneumonia. Some other issues examined included concordance between macroscopic estimation of coronary artery narrowing and microscopic examination, with only moderate agreement. He stated that histology was regarded as in some way contributory to the cause of death in 53% of cases (that is providing, altering, or confirming the cause of death). In 203 cases where the cause of death was not given after macroscopic examination, histology provided the cause of death in 49 cases (24%). The major discrepancies between macroscopic and microscopic examination were in blocks of the heart and lungs.

Image 1: Vacuolation of cells in the pituitary in hypothermia (H&E, x200).

In a study in 2010, de la Grandmaison et al. examined 1786 autopsies prospectively from Garches, France (9). They did extensive sampling including neuropathology and two pathologists reviewed the slides of 428 randomly selected cases. They excluded sudden infant death syndrome (SIDS) cases and cases in which organs had been retrieved for transplantation, along with skeletonized cases. The population was aged 5-91 years with a mean age of 46.2 years. Natural deaths formed the largest group ($n = 130$), with 113 cases of suicide, 104 accidents, and 40 homicides. The commonest cause of death was cardiovascular disease. In 36 cases (8.4%), only histology established the cause of death. Ischemic heart disease was diagnosed

in nine cases, acute myocardial ischemia in six cases, cardiomyopathy in five cases, and myocarditis in six cases. Acute pneumonia was found in six cases. They stated that mechanism of death was found in 40% of cases and that histology affected the manner in 13% of cases. Traumatic lesions were better documented in 22% of cases. They stated their results of the utility of histology accorded with those of Langlois.

In an examination of 100 cases of hanging, incidental findings were made on histology, but microscopic examination did not provide any contribution to the mechanism, cause, or manner of death (10)

Image 2: Soot in airway in fire death (H&E, x100).

Fronczek and colleagues examined 500 coronial autopsy cases (11). In their cases, they had 113 natural deaths, 65 homicides, 60 suicides, 160 accidents, predominantly road traffic accidents, complications of medical therapy in seven cases, and 95 deaths were classified as undetermined. The mean age of the cases was 49 years. Histology was retained in 287 cases. They found that in these 287 cases, five (2%) were diagnosed by histology alone. In 8% of cases, the histology added to the medical cause of death. In 61%, it confirmed the cause of death and in 30% histology played no part in determination of cause of death. Discrepancies were found between macroscopic and microscopic findings in 16% of cases.

Overall, the published studies on the utility of histology in forensic pathology practice show variable results and reflect the different practices that occur in different medicolegal jurisdictions. It is well recognized that there are significant differences in autopsy rates between countries (12) and, therefore, it is not surprising there are different rates for the role and value of histology in determining the cause of death.

A debate in the journal *Forensic Science, Medicine and Pathology* discussed the value of forensic histology (13-18). The authors were all experienced forensic practitioners. In general, the authors were in favor of the use of histology, with some strongly advocating for more, not less histology. The main counter argument is for professional judgment based upon the nature of the case. Published standards may vary between jurisdictions and the authors also pointed out the need to follow the legal requirements, which may restrict the ability to take histology.

Histology

Histology is the microscopic structure of tissues and histopathology can be defined as the microscopic changes in tissue caused by disease (1, 2). Descriptions of procedure and stains used are contained in standard textbooks (2). Briefly, the standard method of producing microscopic slides is to take tissue and place it in a fixative, most commonly formaldehyde solution (formalin) (2). The concentration is normally 4% formal-

dehyde solution, formaldehyde being 40% and then diluted ten times, which is why it is more commonly referred to as 10% formalin solution. Twenty percent formalin is often used in the fixation of brains. Formaldehyde can be buffered to give a neutral pH of 7 with a phosphate buffer. Time to fixation depends on the tissue and its size, with small fragments fixing in four to six hours. Once tissue is fixed, it can start the process of being converted into a microscopic slide by a process of dehydration in ethanol, typically with a small amount of methanol added. This is followed by clearing, typically with a solution like xylene or toluene. It is then impregnated with paraffin wax and embedded to allow cutting on a microtome and the production of a slide, a process known as microtomy. Today, these processes have variable degrees of mechanization, but some processes such as microtomy are still labor intensive. Once a tissue slide is produced, it can be stained with histochemical or immunohistochemical stains. An alternative to paraffin embedding is to perform frozen sections (2). This allows rapid diagnosis but with inferior histology. However, it is required for detection of certain substances that are removed in the paraffin embedding process, notably fat stains in forensic practice.

Histochemical Stains

The most widely used stain in microscopy, including in forensic pathology practice, is hematoxylin and eosin (H&E) (2). Hematoxylin is derived from the tree *Hematoxylon campechianum* and stains nuclei blue-black. A variety of hematoxylin exist (2). Hematoxylin is not a stain, but its major oxidative product, hematin, is. Eosins are xanthene dyes and stain the cytoplasm pink-red. Some laboratories use other stains such as hematoxylin phloxine saffron (HPS), though this stain is more expensive (2).

The normal procedure is to stain all sections taken with H&E and then additional stains as required. In forensic practice, there are a limited number of histochemical stains used in regular practice (**Table 1**). These include connective tissue stains, iron stains, membrane stains, and stains for microorganisms.

Connective Tissue Stains

A number of connective tissue stains are available including Van Giesson, Masson trichrome, Martius scarlet blue (MSB or Lendrum's stain), phosphotungstic acid hematoxylin (PTAH), periodic acid-Schiff (PAS), methanone silver, and reticulin (2).

Masson trichrome, a commonly used stain in our practice, stains nuclei blue-black, cytoplasm red, and collagen blue (**Image 3**). Another useful stain is Movat's pentachrome. This stains collagen yellow, mucin blue, fibrin an intense red, muscle red, and elastin black. It thus has great utility in investigating vascular tissue (**Image 4**). Elastin is highlighted in other stains, such as Verhoeff's method. Martius scarlet blue is useful for staining fibrin. Periodic acid-Schiff (see below) is useful for staining basement membranes and tissue carbohydrates (2).

Periodic Acid-Schiff Stain (PAS)

Periodic acid-Schiff stain, commonly referred to as PAS, is a useful stain for the identification of glycogen and is therefore helpful in glycogen storage diseases. Glycogen can be removed with pretreatment with diastase. It can be positive in hepatocytes in alpha-1 antitrypsin deficiency where intrahepatic globules stain with PAS and are diastase resistant. Periodic ac-

id-Schiff stains fungi, though only viable hyphi are stained (Grocott stains both living and dead hyphi and basement membrane - see below), such as in the kidney (2). The PAS stain is useful in identifying glycogen in the Armanni-Ebstein lesion, which can occur in diabetic ketoacidosis (**Images 5 and 6**).

Iron Stain

Iron is seen following hemorrhage with a period of survival. It may also accumulate in cirrhosis of the liver and hemochromatosis. Dating of injuries can be helped by the presence of hemosiderin, which takes 48-72 hours to be present (**Images 7 and 8**). Hemosiderin is visible on H&E stained slides but highlighted by Perls' Prussian blue stain. Perls' method is considered the first classic histochemical reaction and the method dates to 1867 (2).

Fat Stains

The demonstration of fat is important in fat embolism, though changes in vessels, particularly in the lungs, can be suggestive of fat embolism on routine staining (**Images 9 and 10**). Fat may also be present in other organs, such as the renal tubules in ketoacidosis and in the liver in a variety of conditions (**Images 11 and 12**). In our practice, we stain the liver, kidney, and myocardium for fat in infant deaths (2).

Table 1: Histochemical Stains Commonly Used In Forensic Histopathology

Stain	Tissue	Example of Case
Hematoxylin and eosin	All tissues	
Masson trichrome	Connective tissue	Fibrosis (e.g., alcoholic liver disease, vascular dissection)
Movat's pentachrome	Connective tissue - elastin highlighted	Vascular dissection
Perls' Prussian blue	Hemosiderin	Aging injury, liver disease
Periodic acid-Schiff	Basement membrane tissue carbohydrates, fungi	Fungi, glycogen
Gram	Bacteria	Bacterial endocarditis, necrotizing fasciitis
Ziehl-Neelsen	Mycobacteria	Tuberculosis
Grocott	Fungi	Fungal infections
Congo red	Amyloid	Senile cardiac amyloidosis, congophilic angiopathy

Fat can be identified by two methods. The first and most common method is to stain fresh, unprocessed tissue. Formalin fixed tissue can be used but must not have been processed as this removes the fat. The frozen section is then stained with oil red O or Sudan black. Osmium tetroxide has post-fixation properties and demonstrates fat well, but has the disadvantage of being toxic and expensive, such that most laboratories use oil red O.

Amyloid

Amyloid is a group of related proteins that may cause human disease and stains for amyloid are occasionally helpful following autopsy. Congo red and Sirius red are stains used to see amyloid, and by polarization amyloid has apple green birefringence. Immunohistochemical stains for amyloid also exist. Amyloid may be present in vessel disease such as congophilic angiopathy (**Image 13**). In senile cardiac amyloidosis,

Image 3: Coronary artery atheroma with plaque hemorrhage and thrombus (Masson trichrome, x25).

staining with congo red is weak and stains better with transthyretin using immunohistochemistry (**Images 14 and 15**) (2).

Microorganisms

Infections are common causes of death. Not all require specific investigation, so the presence of pneumonia or meningitis can be demonstrated macroscopically in most cases and microbiological investigations can identify the causative organism(s). When bacteria need to be demonstrated in tissue, a routine Gram stain is used (2). Fungi can be demonstrated with a PAS or Grocott stain, the later also stains such organisms as *Pneumocystis jiroveci* (**Images 16 and 17**) (2). Mycobacteria can be stained with a Ziehl–Neelsen stain (2)

Immunohistochemistry

Immunohistochemical techniques were developed in the 1970s (2). In forensic practice, it has had more limited use in comparison with surgical pathology (see **Table 2**). However, certain tests have specific forensic application such myoglobin to diagnose myoglobinuria in rhabdomyolysis (**Image 18**) and amyloid precursor protein (APP) to identify damaged axons. CD 68 stains macrophages and can provide information on dating injuries. Tumor markers may help in determining the type of cancer present, but usually these have limited medicolegal value, though there might, in occasional cases, be genetic implications. Cytokeratin immunohistochemistry is very useful in diagnosing amniotic fluid embolism (**Images 19 and 20**)

Image 4: Carotid artery dissection (Movat stain, x100).

Table 2: Some Immunohistochemical Stains Used In Forensic Pathology Practice

Stain	Tissue	Example of Case
Myoglobin	Myoglobin	Myoglobinuria
Amyloid precursor protein (APP)	Damaged axons	Head injury, cerebral hypoxia
Cytokeratins	Multiple	Undifferentiated cancer, amniotic fluid embolism
Amyloid	Multiple	Cardiac amyloid
CD 68	Macrophages	Dating injuries

Image 5: Armani-Ebstein lesion in the renal tubules (H&E, x200).

Decomposed Tissue

Although autolysis and putrefactive changes can have significant adverse effects on the quality of histology, it is still possible to find relevant changes when these changes are not too advanced and use of histology in decomposed bodies may be of value (**Image 21**). Rajapaksha and Pollanen, for example, describe a case of liver emboli to the lung in a decomposed body indicating hepatic trauma in life (19).

Cost of Histology

The cost of conducting routine histology cannot be ignored by medical examiner offices and forensic pathol-

ogy units. Costs are inevitably going to vary between and within countries and may depend on whether the office has a standalone laboratory, is part of a large hospital laboratory, or whether histology is sent out to another facility. In our region, the cost of preparing a paraffin block and a single H&E slide is around \$30-40 (Canadian). A histochemical slide costs \$20 and immunohistochemistry stain around \$30. We typically take three to five blocks per case, routinely examining myocardium, lung, liver, and kidney. An average of three to five blocks with just H&E staining will cost between \$90-200 Canadian per case. If four blocks of histology were taken in every case for a pathologist performing 200 cases per year, assuming \$30 per block, this would mean costs for histology per pathol-

Image 6: Armanni-Ebstein lesion in the renal tubules (PAS, x200).

ogist of around \$24 000 Canadian before any special stains or extra blocks were ordered. These are not inconsiderable amounts of money for a cash starved office. To this must be added the issues of cost and security of long-term storage. Currently, our policy is to retain blocks on medicolegal cases for 50 years.

CONCLUSION

Microscopic examination of tissues in forensic pathology practice clearly has utility. In comparison with surgical pathology, a smaller panel of stains is needed. There is, however, an ongoing debate about

whether to take histology on every cases or just select cases based on the available information. Clearly, taking histology in every case has significant resource implications, both for the costs and time of producing slides and the pathologist's time to read them. An opposite view can be put forward that histology in every case provides confirmation (or refutation) of the macroscopic diagnosis. Histology also provides reviewability of the diagnosis and is essentially a permanent record. Furthermore, the examination of routine tissues does provide a continuing experience for the pathologist so they do not lose their skills and also acquire knowledge looking at normal variants.

Image 7: Skin bruise in child (H&E, x100).

Image 8: Skin bruise in child (Perls', x200).

Image 9: Lung. Fat embolism (H&E, x50).

Image 10: Lung. Fat embolism (oil red O, x50).

Image 11: Kidney with subnuclear vacuolation in ketoacidosis (H&E, x50).

Image 12: Kidney with subnuclear vacuolation in ketoacidosis due to fat accumulation (oil red O, x25).

Image 13: Congophilic angiopathy (Congo red, x200).

Image 14: Senile cardiac amyloidosis (H&E, x100).

Image 15: Senile cardiac amyloidosis (transthyretin immunohistochemistry, x100).

Image 16: Lung with pneumocystis pneumonia (H&E, x100)

Image 17: Lung with pneumocystis pneumonia (Grocott, x100).

Image 18: Kidney. Myoglobin in rhabdomyolysis (myoglobin, x200).

Image 19: Lung. Amniotic fluid embolism (H&E, x100).

Image 20: Lung. Amniotic fluid embolism. Immunohistochemistry for cytokeratins (AE1/AE3, x200).

Image 21: Decomposed lung showing features of crack cocaine use with prominent black areas (H&E, x25).

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