A New Resource for Integrated Anatomy Teaching: The Cadaver's Heart PG (Pathology Guide)

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Abstract
The purpose of this project was to develop guides to assist in the examination of a fixed cadaveric hearts and evaluate their effectiveness for assisting students in identifying pathologic lesions. A thorough anatomic examination was performed on 36 cadaveric hearts to determine the "normal" characteristics of an embalmed cadaveric heart and to identify gross pathology. Comprehensive guides with detailed images were prepared and disseminated into two gross anatomy courses, one in the medical school and the other in nursing. Both student groups were assessed to evaluate the effectiveness of the guides as an educational resource. Students who used the guide had a significantly better ability to identify pathology in a cadaveric heart than students in the control group. Implementation of this guide in the gross anatomy laboratory will advance the educational utility of the cadaver. It will also assist faculty and students to identify more learning opportunities from cadaveric tissue that is ultimately sourced from willed-body donors. doi: 10.21692/haps.2017.050

Key words: heart pathology, cadaver, gross anatomy, pathology, anatomy education

Introduction
Pathology found in the gross anatomy laboratory has been reported in the literature to demonstrate how a pathologic occurrence can be an integrative learning opportunity and to report the types of pathology that are identifiable in the cadaver "population." For example, the pathological occurrence of an abdominal aortic aneurysm has been documented to serve as a model for using the cadaver as an integrative teaching tool (Alyafi *et a*l. 2012).

One of the shortcomings of the current literature on pathology in the gross anatomy laboratory is that it is variable in scientific rigor. One factor that varies is whether the pathology is confirmed via microscopy or whether pathological observations are documented using only gross documentation. For example, intra-abdominal pathology in thirty-six cadavers was reported from gross observations only (Magril *et al*. 2008). In another example, atherosclerosis was found to be the most common pathology detected by gross inspection of 50 hearts in the gross anatomy laboratory (Chun *et al*. 2006). However, none of the lesions were verified via microscopy. Wood *et al*. (2012) screened twelve cadavers for the prevalence of pathology. These researchers admitted to having short health histories for each of the cadavers in the study, but none of the pathology reported was confirmed via microscopy. The omission of histologic confirmation may be an issue because it is uncertain how the embalming process alters the tissues of the cadaver.

Although the cadaver is becoming the centerpiece of horizontal and vertical integration in some institutions, proper documentation of the prevalence of certain pathologic lesions within the cadaver "population" is still in its infancy. It would be useful for students and faculty to know which pathologic lesions are commonly encountered in the gross anatomy laboratory so that they can take advantage of their presence. Also, if educators knew which types of pathology were most prevalent, they could prepare themselves if they are not previously familiar with those pathologic concepts. The purpose of this project was to survey cadaveric hearts for gross and microscopic characteristics of pathologic lesions and create a reference guide to illustrate common pathologic lesions as well as artifacts of the embalmed cadaveric heart. After creation of the guide, we aimed to show that these guides were effective resources for teaching students to evaluate the cadaveric heart for grossly observable pathologic lesions.

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Methods

The Institutional Review Board (IRB) of Louisiana State University Health Sciences Center deemed the protocol exempt from IRB oversight (IRB# 8406). .
Thirty-nine cadaveric hearts were obtained from the gross anatomy laboratory after completion of the dissection process.

Data Collection for the pathology Guide

Gross evaluation of the organs
The heart was weighed and the weight in grams recorded. Beginning at the superficial aortic wall, the right coronary artery was cut in cross sections every 0.3-0.5 cm to identify areas of stenosis or occlusion as well as concentric or eccentric narrowing. Then, the procedure was repeated for the left main coronary artery, left descending artery and the circumflex artery. The site and percentage of stenosis was recorded as well as the presence or absence of calcification. Then the heart was serially sliced in 0.5 cm cross sections until the inferior margin of the atrioventricular valves was reached. The myocardium was visually examined for lesions. The size, location (subendocardial or transmural) and characteristics of any myocardial lesions were recorded. The thickness of the left ventricle, right ventricle and interventricular septum were measured.
The right atrioventricular valve (tricuspid) was measured using a flexible tape measure. The valve cusps were examined for abnormalities and calcifications. These steps were repeated for the left atrium, left atrioventricular valve (mitral), and aortic valve.

Histopathologic examination of organs
Samples of tissue were taken from the myocardium of the left ventricle, right ventricle and interventricular septum as a representative of "normal" tissue sections. Then, separate sections were taken from areas where lesions were identified. The tissue cassettes, disposable plastic containers used to hold and identify tissue samples, were processed and embedded with paraffin wax using a standard overnight protocol using an Excelsior ES tissue processor. After placing the tissue in paraffin blocks using a Shandon Histocentre 3, the tissue was sectioned at five micrometers using a Leica RM 2135 microtome and manually stained with Hematoxylin and Eosin. Two board certified pathologists, each with over 10 years of experience, along with a senior pathology resident, examined the slides. The clinicians were blinded as to the gross observations that were described with each case.

Methods of qualitative analyses

All of the gross observations were placed in categories using a qualitative content analysis approach that included an open coding process followed by a selective coding process. Content analysis is a method of qualitative research where words are coded, grouped and recoded to identify themes that exist within documents. For this process, an anatomist (PhD trained) with experience in qualitative research methodology read the written notes that were compiled after the gross and histologic evaluations. The documents were then coded in a two-phase process as described below.

The open coding process
Each individual raw observation was taken from the notes made at the time of gross examination of the heart. The observations were listed in their original language. Then, they were grouped together based on common themes and attributes. For example, individual categories such as "black lines" and "black spots" were grouped together into one category, "black lines and spots". There were three rounds of grouping until the categories could not be condensed without losing their individuality. For example, "black lines and spots" would not be grouped together with "white lines and spots" because this would not allow an analysis between white and black discoloration of the parenchyma.

Selective coding process
After the grouping of observations, the list of categories was considered to be the proposed selective code. Then, using the selective coding system, the notes of the gross observations for each organ were re-read and the observations were placed in one of the categories from the selective code system. Saturation was achieved when all observations fit into a category and no additional categories were needed for completion of the analysis. The same coding process was used for the microscopic observations made by the pathologist.

Determination of cardiomegaly and hypertrophy

The gross observation of cardiomegaly was determined by a heart that has a weight over 436 grams for a male and 390 grams for a female (Grandmaison *et al*. 2001). The parameters for ventricular hypertrophy were a left ventricular thickness greater than 1.5 centimeters (cm) or a right ventricular thickness greater than 0.5 cm. These categories (cardiomegaly and hypertrophy) were added to the aforementioned selective coding system to allow for a correlation analysis.

A Phi correlation (φ) analysis was conducted among all categories in the selective coding systems (both gross and histologic) to determine statistical significance of correlations between the categories. This statistical analysis was conducted using the Graphpad Prism 6 software program. All correlations with a p value less than 0.05 were considered significant.

Development of the cadaver pathology guide

After statistically analyzing all of the correlations between the gross and microscopic observations, a list of significant correlations was generated. The list was then analyzed based on basic pathologic principles to determine whether any of the correlations were indirect, or artificially created by the categorization process; those items were removed from the results. The remaining correlations were considered

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of importance for creating the pathology guide (Table 1). There were also statistically significant correlations between cardiomegaly and the other gross and histologic observations. Dilation of the ventricle, cardiomegaly and ventricular hypertrophy were all positively correlated with gross or histologic observations.

Table 1. Significant correlations between gross and microscopic characteristics

Gross observation	Histologic observation	phi coefficient
Bypass grafts	Scars	(rφ = .456)
Conc. Tan streaks	Scars	(rφ = .649)
Diffuse tan streaks	Interstitial fibrosis	(rφ = .431)
Hemorrhage	Narrowing of arteries	(rφ = .480)
Red streaks in myo.	Recent infarct	(rφ = .466)

Table 1. This table lists the significant correlations between the gross and histologic observations. The phi coefficient is used to detect an association between two variables. All of the significant correlations were positive, meaning that if one variable was present there was an association of another variable being present. Values represent a weak (0.3-0.5), moderate (0.5-0.7) and strong positive correlation (0.7-1.0).

Interpreting the results of the statistical analysis

The correlation between gross and microscopic observations suggested that those two observations occurred together but did not necessarily suggest any causative relationship between them. The clinically or diagnostically important correlations can do two things: assist an examiner in identifying likely microscopic observations that would be present if the gross characteristic is observed and support the current theories in the field of pathology that those gross and microscopic findings are related to each other. There are also generalizations that can be made as to the lack of correlations between gross and microscopic findings. For example, there was a high prevalence of fat observed grossly in the organs (43.59% of the organs had this gross observation). However, this observation did not correlate with any microscopic finding or other gross lesions. It may be helpful for an examiner to know that the gross observation of increased fat within the ventricular myocardium does not depict or predict the presence of any pathologic state.

Correlations that were determined as having diagnostic or clinical importance are seen in Table 1. They were included in the pathology guide because the gross observation that the heart had evidence of bypass grafts was positively correlated with the microscopic presence of scarring. This correlation is clinically important because the presence of scarring indicates that the heart has had past ischemic injury. As in cases of myocardial infarction, myocytes die when they are permanently deprived of oxygen (Kumar *et al*. 2014). Dying myocytes release their cytoplasmic components into the extracellular environment and this stimulates an inflammatory response marked by the presence of neutrophils. Therefore, the presence of neutrophils and myocytes that lack nuclei is the hallmark sign of necrosis (Figure 1).

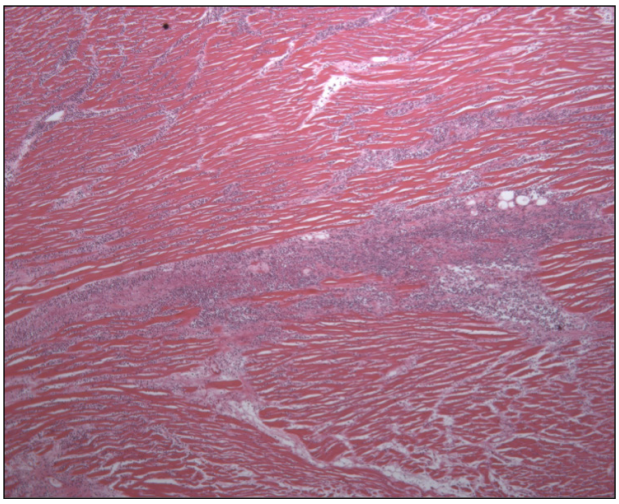

Figure 1. Necrosis of the myocardium
This photomicrograph shows a necrotic region in the myocardium.

These histologic signs were seen in hearts that had recent myocardial infarcts when death occurred before the healing process could be completed. After the neutrophils have been recruited to the area, macrophages respond by migrating into the extracellular space and begin removing cellular debris (Kumar *et al*. 2014). This is followed by a transitional stage of healing that involves the stimulation of new tissue, not myocytes. Fibroblasts proliferate and angiogenesis is stimulated. There is often edema in the area and this transitional phase is collectively called granulation tissue (Figure 2). The stage of granulation tissue will develop gradually into an avascular fibrotic scar, composed mostly of collagen from fibroblasts. For this reason, scarring is evidence of past injury that also could lead to interventions such as stents and/or coronary bypass grafts.

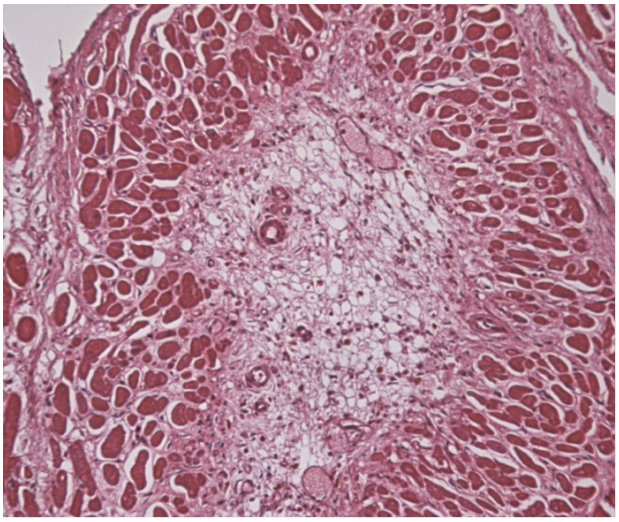

Figure 2. Granulation tissue of the myocardium.
This photomicrograph demonstrates granulation tissue with angiogenesis and edema (center).

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Red streaks and spots in the myocardium were positively correlated with the histologic observation of a recent myocardial infarction. This is important because it deviates from the standards of the field of pathology, where recent myocardial infarctions appear pale or yellow, sometimes with a red ring around the parameter (Virmani *et a*l. 2001, Riede 2004, Kumar *et al*. 2014). This gross observation in fixed cadaveric tissue may be of assistance to individuals who are inspecting organs without the ability to histopathologically confirm the suspicion of a recent myocardial infarct.

The gross observation of concentrated tan streaks in the myocardial wall was positively correlated with the microscopic observation of scars. This is important because the heterogenous appearance of the myocardium, especially white or tan streaks, is usually an indication of scarring in fresh tissue (Kumar *et al*. 2014). This observation was further confirmed in our analysis of embalmed tissue. However, the separation had to be made between "concentrated tan streaking" of the myocardium and "diffuse tan streaking" of the myocardium for this correlation to be apparent. When the heterogeneity was grouped together, it was not correlated with scarring.

The presence of diffuse tan streaking was positively correlated with the microscopic observation of interstitial fibrosis. This observation is usually not made in fresh tissue. However, in embalmed tissue, the interstitial fibrosis causes a streaking pattern that is diffuse and can be misinterpreted as replacement fibrosis (Figure 3). Therefore, the separation between concentrated and diffuse tan streaking should be made first in embalmed hearts before the determination can be made about the presence of scarring or interstitial fibrosis.

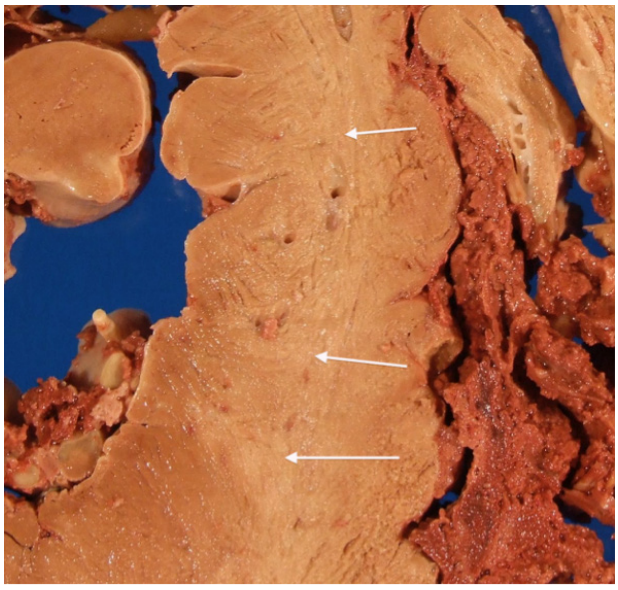

Figure 3. Discoloration of the myocardial tissue.
Diffuse tan discoloration of the myocardium. This is not correlated with scarring of the myocardium, but rather interstitial fibrosis in 50% of incidences.

The gross observation of hemorrhage was positively correlated with microscopic narrowing of the coronary arteries. This correlation is clinically important because eccentric stenosis, as a result of atherosclerotic plaques, can be structurally unstable (Kumar *et al*. 2014). Atherosclerotic plaques are generated from the progressive deposition of lipids in the arterial wall. Endothelial cells and macrophages attempt to remove this extracellular lipid; consequently lipids accumulate in the cytoplasm. As the disease progress, a fibrotic cap, produced by smooth muscle cells and foam cells (endothelial cells and macrophages that have ingested lipid), cover the extracellular lipid deposition. The thickness of this cap is important in determining the stability of the plaque (Abdelai *et al*. 2014). The plaque commonly protrudes into the lumen of the vessel and can result in reduced blood flow and turbulent blood flow. Both of these consequences can add insult to the compromised tissue depending on the severity of the narrowing. If the fibrotic cap is thin or the individual has other complicating factors, such as hypertension, the plaque can rupture and lead to hemorrhage or thrombus formation. Although hemorrhage can be caused by other factors such as trauma and hypertension, the casual relationship between narrowing of the arteries and hemorrhage can be made and explained in a clinical context.

Dilatation of either ventricle was positively correlated with cardiomegaly. This correlation is expected considering that dilation is one of the gross characteristics that may be seen in cases of cardiomegaly (Virmani *et al*. 2001, Kumar *et al*. 2014).

Dilatation of either ventricle was positively correlated with interstitial fibrosis. Heart enlargement occurs because of the longitudinal or transverse enlargement of the myocytes within the myocardial wall, and by the increased production of collagen in the interstitium (Virmani *et al*. 2001, Donekal *et al*. 2014). Therefore, the correlation between dilatation and interstitial fibrosis is not surprising.

Dilation of either ventricle was positively correlated with the right ventricular chamber being visible in the first cut when sectioning the heart. This correlation is expected because the right ventricle being present in the first cut of the heart is an indication of right ventricular dilitation (Waller 1988). This dilatation may be a symptom of right heart failure and is frequently due to increased pulmonary vascular resistance (Gollapudi *et al*. 2005). Clinically, this condition is called *cor pulmonale*.

Cardiomegaly was positively correlated with eccentric stenosis of the coronary arteries. This correlation is not as directly linked as the aforementioned ones, but may simply be indicative of the fact that a diseased heart is more likely to demonstrate anatomic alterations such as cardiomegaly. Considering that stenosis and atherosclerosis are predisposing factors to many cardiac diseases and ischemic injury (Ali *et al*. 2015), it is not surprising that this correlation was significant.

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Left ventricular hypertrophy was positively correlated with the right ventricle being in the first cut while sectioning the heart. As previously noted, the right ventricle being in the first cut is a sign of right heart dilatation. Left ventricular hypertrophy occurs in situations where the cardiac output is insufficient and the heart is compensating for this deficiency (Waller 1988, Kumar 2014). If cardiac output from the left ventricle is not sufficient, the backup of pressure can commonly extend to the pulmonary vascular system and to the right side of the heart. As previously suggested, this increase in pressure in the right ventricle can cause anatomic changes such as hypertrophy and dilatation. Left ventricular hypertrophy was positively correlated with red discoloration in the myocardium. Left ventricular hypertrophy is an anatomic alteration that occurs as a compensatory mechanism to increased stress and an increased workload (Virmani 2001, Kumar 2014). Individuals with these cardiovascular conditions are predisposed to acute coronary injury such as myocardial infarctions. Left ventricular hypertrophy was positively correlated with cardiomegaly. This is an expected correlation considering that hypertrophy is a form of cardiomegaly.

Overall, the correlations that cardiomegaly, ventricular hypertrophy and ventricular dilatation had in the embalmed tissue were congruent with the findings that have been accepted in the field of pathology from tissues taken at autopsy. It was not certain whether the embalming procedure would cause artifacts such as dilatation of the left ventricle. The applied pressure of the fixative solution would first enter the aorta and left ventricle because the ligature site for all of the cadavers was the right common carotid artery. However, the diagnostic rule of using weight to determine cardiomegaly correlated with hypertrophy and dilation in a pattern similar to those observed in fresh tissue. No unusual increases in cardiomegaly, hypertrophy or ventricular dilitation were found in this survey. Therefore, the results indicate that the embalming process probably does not induce artifacts such as dilatation or hypertrophy.

From the data obtained during the gross and microscopic observations, the following categories were included in the final version of the pathology guide:

- The size and weight: cardiomegaly and the shape of the heart
- The amount of fat: fat on the outside of the heart and interspersed within the myocardium; right ventricular dysplasia
- The coronary arteries: gross patterns and degree of atherosclerosis
- Valvular pathology: aortic stenosis, valvular thickening and mitral valve prolapse
- The color of the myocardium: tan streaks in the myocardium, red spots and regions in the myocardium
- The thickness of the myocardium: hypertrophy and dilation of the ventricular wall

Evaluation of the Guide's Effectiveness

Content validation of the guide was performed by pathologists (n=3) and students (n=6; three second semester nursing students and three second year medical students). The guides were reviewed for the following categories: clarity in wording, relevance of items, use of standard English, absence of biased words or phrases, formatting of items, clarity of instructions (Fowler 2002). The content validation forms were qualitatively analyzed to identify themes in the reviewers' comments. The major suggestions were to add word definitions to the beginning or end of the guide for selected terms and to elaborate on the instructions. Reviewers unanimously commented that the guides would be helpful in the gross anatomy laboratory for both of the target populations.

Assessment of the effectiveness of the cadaver pathology guide

At Louisiana State University Health Sciences Center, both medical and nursing students take a gross anatomy laboratory that involves cadaver dissection. In addition, both student populations take a course in pathology in their second year of study. After the students completed dissection during their gross anatomy course, but before they were enrolled in pathology, students were recruited to participate in a short educational intervention to measure the effectiveness of the pathology guides as a teaching resource. Medical (n=87) and nursing (n=84) students were asked to use the guides to identify pathology when examining cadaveric hearts. Cadaveric specimens of the heart were arranged at six assessment stations in the laboratory (Figure 4). For each specimen, students were asked to evaluate the organ and determine if the organ had any signs of pathology based on the specimen's gross anatomical characteristics. Half of the students used the provided pathology guide to assist them in their evaluation of the organ (experimental group) and half of the students had a laboratory guide that only included definitions of the pathologic terms (control group).

The control group scores were compared to the experimental group scores by performing a t-test using Graphpad Prism 6 software program. Effect size was estimated by calculating Cohen's d. The internal consistency of the assessment was determined by calculating KR-20.

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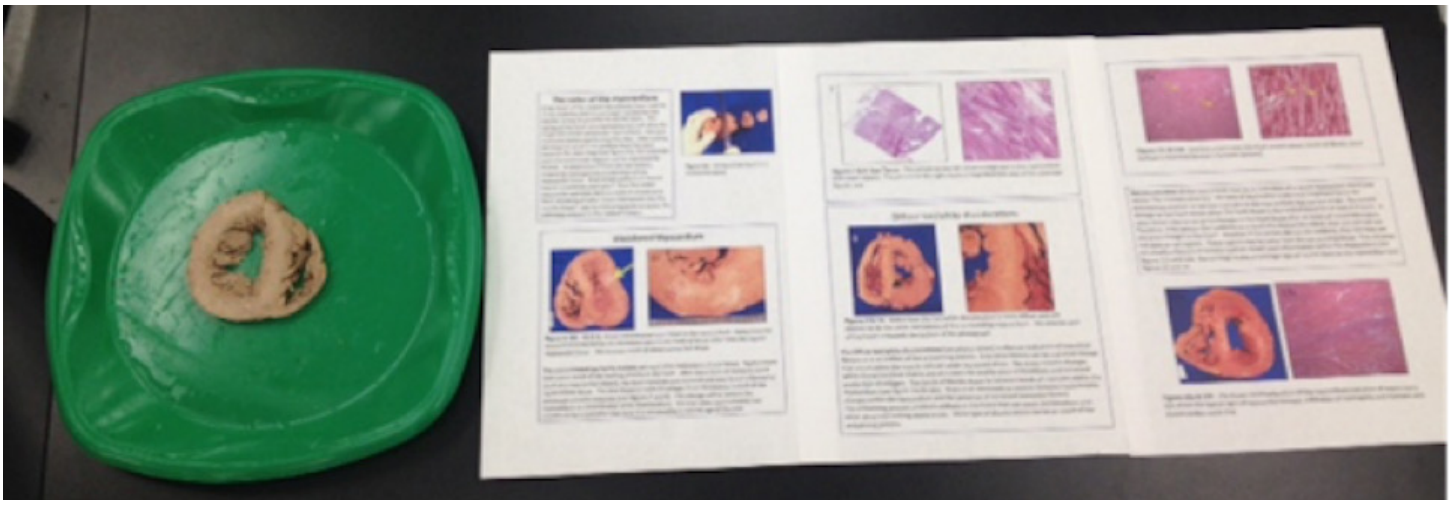

Figure 4. Assessment station.
This figure shows an example of a heart assessment station. The cadaveric specimen is on the left. Student participants could use the guide provided on the right to determine if the specimen had any signs of pathology.

Results

The group using the pathology guide, overall, had a significantly higher average percentage of items correct than the control group in both student populations (Figure 5). For the nursing population, the percentage correct was 40% for the control group and 60% for the experimental group. For the medical student group, the percentage correct was 53.3% for the control group and 73.3% for the experimental group. The internal consistency of the assessment was calculated and was KR-20= 0.50, which is a good level for an instructor-made assessment. The results suggest that the pathology guide assisted the students in accurately identifying significantly more pathologic changes within cadaveric tissues even within a short period of time (20 min).

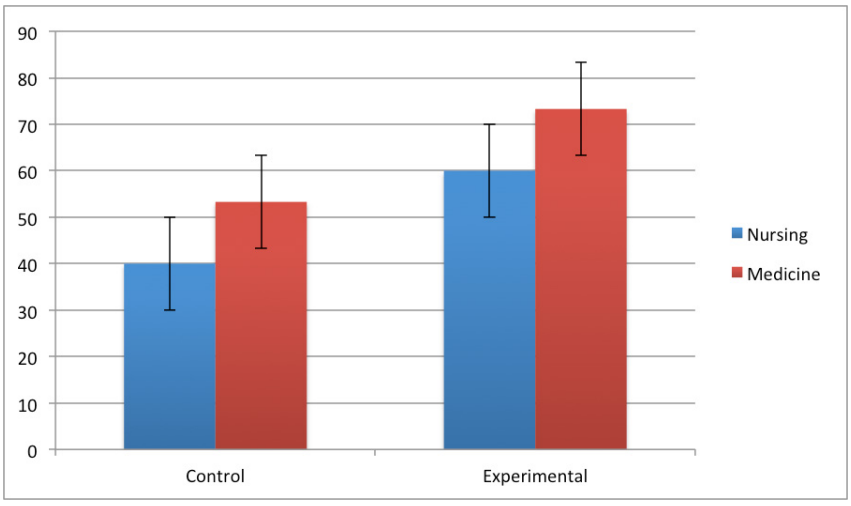

Figure 5. Scores from assessment for nursing and medical student populations.
In both populations, the experimental group was significantly higher than the control group, $p<0.01$. Values represent the percent of the total number of questions the student got correct.

Discussion

This pathology guide is the only existing resource that we are aware of that demonstrates the extent of gross characteristics that is an indication of pathologic lesions detected in embalmed cadavers. Most of the pathology guides that exist show depictions of pathologic lesions and anatomic evidence of disease processes in unfixed, autopsy patients. The impact that these guides will have on the health professions field is in educating both teachers and students about how common disease states appear in cadavers used within a gross anatomy course. Most health care practitioners participate in cadaveric dissection in their training programs. It is our goal to assist these students to undertake self-directed learning by recognizing common pathologic presentations of cadaveric organs.

Our guide was based on the statistical analysis performed after an anatomic study of cadaveric organs, both on the gross level and the microscopic level. This in-depth process allowed us to single out gross characteristics that were artifacts caused by the embalming process. It is our hope that clinicians and other educators, such as anatomists who teach in gross anatomy laboratories, can also use these guides to identify artifacts in the cadaver "population".

Overall, these guides aim at increasing the educational value of the cadaver, thereby increasing the impact of the anatomic gift (body donation).

This resource is limited in the fact that there are many pathologic processes that cannot be observed grossly and require histology for confirmation. We added histologic correlations in our guide to allow for histopathologic

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confirmation at institutions with these resource capacities. All histologic representations were also made from the embalmed cadaveric tissue from the gross anatomy laboratory.

The authors would like to acknowledge the individuals who donated their bodies for the advancement of science. Their contribution made the development of these resources possible.

The Cadaver Heart PG guide is freely available for download on LSUHSC's website at: http://virtualhumanembryo.lsuhsc.edu/GIFT/PathGuides%20-%20Copy.html.

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