Simplification of Necropsy Observation in the Pharmaceutical Industry

James H. McGee,
DVM, DABT

Scientific Director,
Preclinical Research
BAS Evansville

email:
jmcgee@bioanalytical.com

Laboratory animals remain an important component of evaluating whether drugs or medical devices are safe and effective, and whether food additives or industrial/agricultural chemicals are safe in the work place or environment. An important component of such studies is the necropsy (autopsy) in which gross observations are made about the anatomy of all organs, especially those commonly indicative of a toxic response to the applied xenobiotic chemical. Prior to the necropsy, a comprehensive evaluation (hematology, clinical chemistry and urinalysis) of a variety of body fluid constituents makes comparisons between control and experimental animals during the course of exposure of the laboratory animals to the test article. The necropsy is followed by a detailed microscopic examination (histopathology) of the tissues for changes in cellular morphology. The histopathological examination thus incorporates the findings from the veterinary clinical pathology and necropsy examinations.

Traditional methods teach that necropsy observations are to be recorded in exquisite detail, always including location, color, size, shape, number, content of cavities, and consistency of every lesion found (1). This was thought to be necessary in order to record all the subtle clues to the disease process that had resulted in the death of the animal. These detailed gross observations were coupled with the microscopic histopathology observed in the tissues collected and sectioned, ultimately leading to a final diagnosis of the disease.

While this may still be necessary in disease diagnosis, recording observations to this degree of accuracy in a typical toxicology study is not practical or warranted. A significant problem in the pharmaceutical industry is the large number of animals used in studies and the necessity to summarize and tabulate necropsy observations for this large number of animals. We are constantly looking for ways to reduce the number of animals used, and the time it takes to assemble the information necessary for drug approval. Refining necropsy methods is one way to help accomplish these goals.

The description of gross lesions becomes particularly important when attempting to summarize a diverse group of changes and when trying to correlate gross findings with the histopathological diagnosis. The issue of correlating gross lesions with histopathological changes is also a regulatory dilemma (2).

To illustrate some of the problems associated with recording gross necropsy lesions, consider the hypothetical file of necropsy records for a typical study in the rat shown in ***T1***. For the sake of brevity, only observations of the liver have been included.

In this example, there are only five rats/sex/group with a control and three dose groups for a total of 40 rats.

The information in the summary table of necropsy observations for the hypothetical data shown in ***T1*** is not representative of the real life situation. The real life situation is complicated even further by the necessity to collect more than 40 tissues and/or organs from each animal. Collecting massive amounts of data does not mean one has accomplished anything worthwhile. Although it is necessary to make observations at necropsy, it is certainly not necessary to make observations with this degree of detail in a toxicology study.

This information is not going to be used by the pathologist to describe the histopathological lesion(s) present in the tissue, and it will not be used to make a diagnosis of drug effect. It is not possible for a pathologist to determine tumor type by its size or color.

Necropsies are still being conducted in this manner because that is the textbook method, and people in management and regulatory positions have come to expect that degree of detail in the reports. In a toxicology study, and for that matter in any necropsy, the purpose of the observations is to prompt the pathologist to pay special attention to certain lesions/organs. As long as there is some type of record to key the attention of the pathologist, the purpose of the necropsy has been accomplished, and management and regulatory personnel will be provided with the information they need to make a decision about the fate of the compound.

In the simplified system developed for pharmaceutical studies, there are only two color options: normal and discolored. Simply stated, if the color is not normal then it must be abnormal. The system shown in ***T1*** results in innumerable colors and color combinations that are impossible to place in a summary table. (If it were possible to summarize all the colors listed, that analysis would not have enough relevance to justify eliminating a compound because the pathologist sees a variety of different colors associated with the liver at necropsy.)

In addition, it is almost impossible to correlate color with a histopathological change in an organ or tissue. In many cases, the color that was apparent at necropsy is no longer apparent at gross cutting because it has been destroyed or changed by the formalin used to fix the tissues. Many times the failure of the pathologist to correlate gross and histopathological changes is related to a futile attempt to associate a specific microscopic lesion with a color that was recorded at necropsy.

Considering the issue of size, size matters only in relation to an entire organ, thus it is important to note whether or not an organ is larger or smaller than normal in order to alert the pathologists to a potential problem. Hence only two sizes need to be considered: smaller or larger than normal.

If the organ appears to be denser than normal on the cut surface, the pathologist will examine the microscopic slide and make a determination about the presence or absence of fibrous connective tissue. The same is true of any other observation recorded related to consistency; however, I believe it is not necessary to describe consistency at all.

T1 and ***T2*** compare the traditional system with the simplified system. ***T2*** contains the same information as ***T1***. In both cases, the pathologist has been given the information needed to relate microscopic examination to the affected tissue or organ part. Since each gross lesion has been isolated and placed in a separate capsule, it is less likely that a gross lesion seen at necropsy will be lost during processing to slides. This also results in a list of lesions that is easy to summarize and interpret.

It is not possible to arrive at a conclusion based on either ***T1*** or ***T2***. Results of the histopathological examination are necessary in order to arrive at any final conclusions about the affects of the drug on the liver during this study.

Summarizing this discussion, the purpose of necropsy observations is to inform the pathologist of potential lesions in a particular organ or tissue. Describing the changes we see at gross necropsy in exquisite detail will not change or influence the final histopathological diagnosis or the final conclusion on development of the drug.

T1: Individual Gross Necropsy Observations, Study No. 1234

Animal Number and Observation

Group I Males

1. There is a 2 mm diameter reddish-brown lesion on the median lobe of the liver. The lesion has the consistency of normal liver and is not raised above the surface.
2. NGL = No Gross Lesions
3. There is a 1 mm x 3 mm reddish-tan area on the caudate lobe of the liver. The area is slightly raised above the surface of the liver and has a consistency similar to normal liver tissue.
4. There is a 0.5 cm diameter grayish-white area on the left lateral lobe of the liver. This area is raised approximately 1 mm above the surface of the liver and has a firm consistency.
5. NGL

Group II Males

6. NGL
7. There is a 1.0 x 2.3 x 1.4 cm lobulated mass on the right lateral lobe of the liver. The mass is greenish-tan in color.
8. There is a raised, brownish-tan lesion on the median lobe of the liver. The lesion is 0.5 mm in diameter and has a consistency similar to normal liver.
9. There is a raised, yellowish-tan lesion on the median lobe of the liver. The lesion is 0.5 mm in diameter and has a consistency similar to normal liver.
10. NGL

Group III Males

11. NGL
12. NGL
13. There is a blackish-tan, 0.3 mm lesion on the right lateral lobe of the liver. The lesion is depressed approximately 0.1 mm below the surface of the liver. The consistency is that of normal liver.
14. There is a 1 mm diameter brownish-red lesion on the median lobe of the liver. The lesion has the consistency of normal liver and is not raised above the surface.
15. NGL

Group IV Males

16. There is a 1.0 x 2.3 x 1.4 cm lobulated mass on the left lateral lobe of the liver. The mass is yellowish-gray in color.
17. There is a 1.0 mm diameter grayish-brown area on the left lateral lobe of the liver. This area is raised approximately 0.5 mm above the surface of the liver and has a firm consistency.
18. There is a 1 mm x 3 mm yellowish-tan area on the caudate lobe of the liver. The area is slightly raised above the surface of the liver and has a consistency similar to normal liver tissue.
19. There is a 1 mm diameter reddish-tan area on the caudate lobe of the liver. The area is slightly raised above the surface of the liver. On cut surface the tissue appears to be denser than normal liver tissue.
20. There is a greenish-tan, 0.3 mm lesion on the right lateral lobe of the liver. The lesion is depressed

approximately 0.1 mm below the surface of the liver. On cut surface the tissue contains cysts containing a clear gelatinous fluid.

Group I Females

21. NGL
22. NGL
23. NGL
24. NGL
25. There is a 0.5 x 1.0 x 1.4 cm lobulated mass on the right lateral lobe of the liver. The mass is grayish-white in color.

Group II Females

26. NGL
27. There is a 1 mm x 3 mm purplish-tan area on the caudate lobe of the liver. The area is not raised above the surface of the liver and has a consistency similar to normal liver tissue.
28. NGL
29. NGL
30. NGL

Group III Females

31. NGL
32. There is a 1.0 mm diameter bluish-gray area on the left lateral lobe of the liver. This area is depressed approximately 0.5 mm below the surface of the liver and has a firm consistency.
33. There is a 1.5 mm diameter red lesion on the median lobe of the liver. The lesion has the consistency of normal liver and is slightly raised above the surface.
34. There is a 1.5 mm diameter brownish lesion on the median lobe of the liver. The lesion has the consistency of normal liver and is depressed approximately 0.5 mm below the surface.
35. NGL

Group IV Females

36. NGL
37. There is a 0.5 mm x 1.5 mm tan area on the caudate lobe of the liver. The area is slightly raised above the surface of the liver. On cut surface the tissue appears to be denser than normal liver tissue.
38. NGL
39. There is a 0.5 x 1.2 x 1.4 cm lobulated mass on the left lateral lobe of the liver. The mass is reddish-brown in color with a green tinge.
40. NGL

T2: Individual Necropsy Observations, Study No. 1234

Animal Number and Observation

Group I Males

1. Median lobe of liver; focal discoloration — Gross Lesion #1 (GL #1).
2. NGL = No Gross Lesions
3. Caudate lobe of the liver; focal discoloration — GL #1.
4. Left lateral lobe of the liver; multifocal discoloration —GL #1.
5. NGL

Group II Males

6. NGL
7. Right lateral lobe of the liver; mass — GL #1.
8. Median lobe of the liver; focal discoloration — GL #1.
9. Median lobe of the liver; focal discoloration — GL #1. Right lateral lobe; mass — GL #2.
10. NGL

Group III Males

11. NGL
12. NGL
13. Right lateral lobe of the liver; focal discoloration — GL #1.
14. Median lobe of the liver; focal discoloration — GL #1.
15. NGL

Group IV Males

16. Left lateral lobe of the liver; mass — GL #1.
17. Left lateral lobe of the liver; multifocal discoloration — GL #1. Left lateral lobe of the liver; mass — GL #2.
18. Caudate lobe of the liver; focal discoloration — GL #1.
19. Caudate lobe of the liver; multifocal discoloration — GL #1.
20. Right lateral lobe of the liver; focal discoloration — GL #1. On cut surface the tissue contains cysts containing a clear gelatinous fluid — GL#2.

Group I Females

21. NGL
22. NGL
23. NGL
24. NGL
25. Right lateral lobe of the liver; mass — GL #1.

Group II Females

26. NGL
27. Caudate lobe of the liver; focal discoloration — GL #1.
28. NGL
29. NGL
30. NGL

Group III Females

31. NGL
32. Left lateral lobe of the liver; focal discoloration —GL #1.
33. Median lobe of the liver; focal discoloration — GL #1.
34. Median lobe of the liver; multifocal discoloration — GL #1.
35. NGL

Group IV Females

36. NGL
37. Caudate lobe of the liver; focal discoloration — GL #1.
38. NGL
39. Left lateral lobe of the liver; mass — GL #1.
40. NGL

References

1. *J.M. King et al., "The Necropsy Book," L.D. Charles, DVM. Foundation, Publisher.*
2. *P.N. Dua and B.A. Jackson, Tox. Path. 16 (1988) 443-450.*