QUALITATIVE and
QUANTITATIVE REPORTING
OF NON-NEOPLASTIC
LESIONS IN TOXICOLOGY
STUDIES

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Preface:

- Reports are written to convey information concerning the pathologic findings in a toxicology study.
- Reports must be complete, accurate and convey the significance of lesions identified in a study.
- The quality of the report is determined by the thoroughness, accuracy and consistency of the pathologist.
- The use of methodologies for semi-quantitating or quantitating pathologic findings.
Topics

- **Qualitative analysis**: quality indicators of pathology
- **Semi-quantitative analysis**: severity grading
- **Quantitative analysis**: image analysis and stereology
- Conclusions
QUALITATIVE ANALYSIS

• Quality indicators of pathology:
  1. Thoroughness
  2. Accuracy
  3. Consistency
Quality indicators of pathology:

1. "Thoroughness," is the identification of all lesions present in a particular organ or tissue, including spontaneous background lesions. Experienced pathologists familiar with background lesions may use a threshold.
Thresholds

What is a threshold?

• Definition: A “threshold” is the level below which, by general consensus, a lesion is deemed insignificant and therefore is not diagnosed

• The term threshold does not apply to the qualitative exclusion of certain changes, e.g. congenital lesions
Example of a Threshold

**Testicular Degeneration:**

- A single syncytial cell within a seminiferous tubule
  - below threshold, ∴ not diagnosed

- Two to three syncytial cells within a seminiferous tubule
  - above threshold, ∴ diagnosis of testicular degeneration
Why Use Thresholds?

- Arguments for:
  - Avoids “lesion overload” in database
  - Results of individual studies not obscured by excess data
  - Accepts concept that certain types of changes may be appropriate or “normal background” lesions
  - Accepts concept that there are quantitatively acceptable limits for normal (for at least some lesions)
Why Use Thresholds?

- Arguments against:
  - Established thresholds may not adequately represent the control animals
  - May mask subtle treatment-related effects
  - Difficult to get pathologists to agree on what is “normal”
  - Certain lesions will be underrepresented in historical control database which may be useful in interpreting study findings
“PRACTICALITY”

• Thresholds can and should be used:
  ✔ Diagnosing all common lesions can overwhelm your data base
  ✔ Undefined thresholds can result in a drift in criteria
  ✔ Thresholds must be “defined” somewhere in the laboratory report
• Quality indicators of pathology:
  2. “Accuracy” is the application of correct terminology when recording observed lesions. Nomenclature of lesions is a matter of definition. Experienced pathologists generally agree as to what terms are to be used.
• Quality indicators of pathology:
  3. "Consistency" occurs when the pathologist consistently applies a specific term to record a defined lesion. It implies that the same diagnostic criterion is being followed each time.
QUANTITATIVE ANALYSIS

- The significance of non-neoplastic lesions can be measured and recorded either:
  1. semi-qualitatively
  2. quantitatively
• Semi-quantitative analysis:
The application of defined severity grades for specific lesions
Severity Grading

- Most non-neoplastic lesion databases incorporate severity grading.
- The “severity” of various lesions is usually determined by the extent of tissue involvement.
- Although severity grading has been used in toxicology studies, there are **no standardized guidelines.**
Why Develop Standardized Guidelines for Severity Grading?

- Arguments for:
  - To identify subtle treatment-related effects that are not incidence based
  - To maintain lesion consistency among different studies
  - To maintain lesion consistency among different pathologists
  - To increase the accuracy of historical control databases
Why Develop Standardized Guidelines for Severity Grading?

• Arguments against:
  ✓ Guidelines may be too inflexible to accommodate different tissues or situations
  ✓ Grading ranges may be difficult to estimate without morphometric measurements
  ✓ Grades may not correlate well with the degree of physiologic alteration
  ✓ Doesn’t take full advantage of the pathologist’s training and experience
Two Examples of Severity Grading Schemes

<table>
<thead>
<tr>
<th>Grade</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 = Minimal</td>
<td>(&lt;10%)</td>
<td>(0-25%)</td>
</tr>
<tr>
<td>Grade 2 = Mild</td>
<td>(10-39%)</td>
<td>(26-50%)</td>
</tr>
<tr>
<td>Grade 3 = Moderate</td>
<td>(40-79%)</td>
<td>(51-75%)</td>
</tr>
<tr>
<td>Grade 4 = Marked</td>
<td>(80-100%)</td>
<td>(76-100%)</td>
</tr>
</tbody>
</table>
Nephropathy: Extent of Cortical Involvement

~25%

~50%

~75%

~100%
Extent of Cortical Involvement

~50%
## 50% Renal Cortex Involvement

<table>
<thead>
<tr>
<th>SCHEME A</th>
<th>SCHEME B</th>
<th>PROPOSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate (40-79%)</td>
<td>Mild (26-50%)</td>
<td>Marked (41-100%)</td>
</tr>
</tbody>
</table>
Grading Scheme: Grade 1 (+1) = Minimal

• Barely noticeable to changes considered so minor, small, or infrequent as to warrant no more than the least assignable grade (0-10%)
• Focal, multifocal or diffusely distributed lesions, this grade is used for processes where <10% of the tissue section is involved
• Hyperplastic/hypoplastic/atrophic lesions, this grade is used when the affected tissue has undergone <10% increase or decrease in volume
Grade 1 Severity / 10% involved
Grading Scheme: Grade 2 (+2) = Mild

- Noticeable but not prominent feature of the tissue
- Focal, multifocal or diffusely distributed lesions, this grade is used for processes where between 11-20% of the tissue section is involved
- Hyperplastic/hypoplastic/atrophic lesions, this grade is used when the affected tissue has under-gone between 11% and 20% increase or decrease in volume
Grade 2 Severity / 20% involved
Grading Scheme: Grade 3 (+3) = Moderate

- Prominent feature histologic feature of the tissue
- Focal, multifocal or diffusely distributed lesions, this grade is used for processes where between 21-40% of the tissue section is involved
- Hyperplastic/hypoplastic/atrophic lesions, this grade is used when the affected structure or tissue has undergone between 21% and 40% increase or decrease in volume
Grade 3 Severity / 40% involved
Grading Scheme: Grade 4 (+4) = Marked

- An overwhelming histopathologic feature of the tissue
- Focal, multifocal or diffusely distributed lesions, this grade is used for processes where between 41-100% of the tissue section is involved
- Hyperplastic/hypoplastic/atrophic lesions, this grade is used when the affected tissue has under-gone between 41% and 100% increase or decrease in volume
Grade 4 Severity / 50% involved
• Quantitative analysis:
  Involves the use of measuring techniques such as “image analysis” and “stereology” to provide numerical values.
Stereology vs. Image Analysis

- Conventional image analysis:
  - Compares objects on slides
  - Counts accurate for slides (if same thickness)
- Stereology:
  - Estimates the number of objects in a tissue
CONCLUSION

• When these qualitative and quantitative factors are applied in preparation of a pathology report, the recorded pathology findings can be interpreted and put into perspective. The use of this approach assures a reader or reviewer that the pathology report meets the highest standards.
“Food for thought”

Grading schemes:

1. Should the same standardized grading scheme be used by all pathologists in order to maintain consistency?
2. Should different grading schemes be used for different tissues?
3. Should different grading schemes be used for subchronic and chronic studies?
“Food for Thought” (cont.)

The concept and use of thresholds:
1. Should thresholds be used?
2. Can undefined thresholds be applied consistently?
1. For thresholds to be applied consistently do they have to be defined?
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REFERENCES

- Wolf (2011) Counterpoint to "Analysis of unbiased histopathology data from rodent toxicity studies (or, are these groups different enough to ascribe to treatment?)". Toxicol Pathol, 39(6):1017-1019.