REVIEW

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Histological Scoring Systems for the Assessment of the Degree of Lung Injury in Rats

Sıçanlarda Akciğer Hasarının Değerlendirilmesi için Kullanılan Histolojik Derecelendirme Sistemleri

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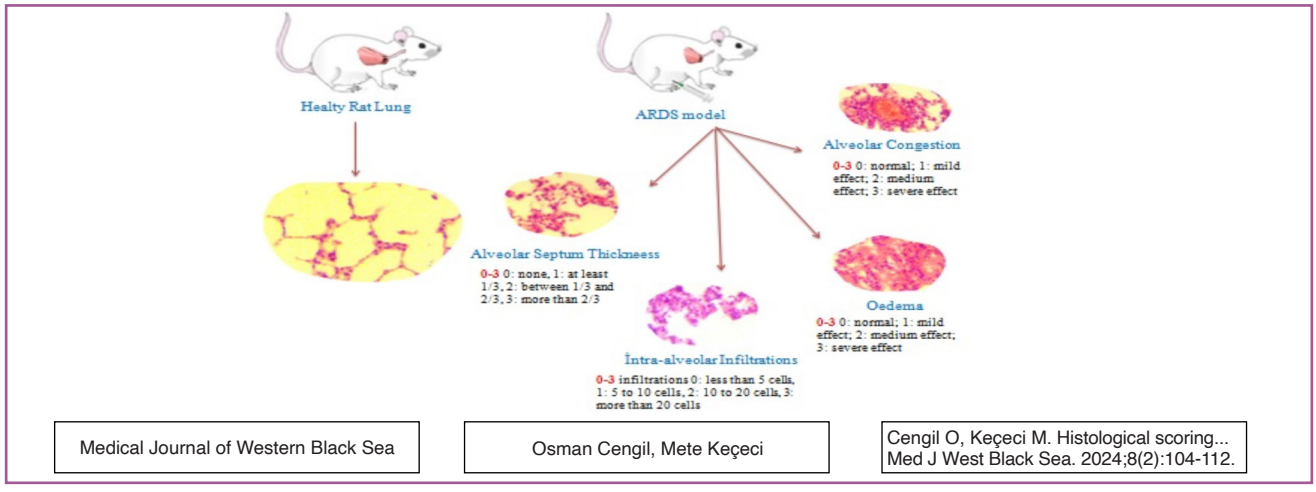
GRAPHICAL ABSTRACT



ABSTRACT

Acute respiratory distress syndrome (ARDS) is a serious pulmonary response with well-defined clinical parameters in humans triggered by many causes besides bacterial and viral pneumonia. However, there is no definitive definition of ARDS parameters in the experimental animal model. With its 2010 workshop report, the American Thoracic Society described the essential histopathological property that determine the entity of ARDS in laboratory animals, such as inflammation, changes in parenchymal tissue, abnormal lung function and altered entirety of the alveolar capillary barrier. Understanding these parameters, scoring tissue lesions is used to convert observational pathological data into semi-quantitative or quantitative data for statistical analysis and improved precision. However, the existence of different animal species and different ARDS experimental models causes confusion in these scoring methods. Therefore, the histopathological lesion scoring systems used for ARDS experimental models have been examined in detail in the studies conducted in the PubMed database in the last five years. The aim of this article is to provide a comprehensive guide to the different parameters and scoring systems used to evaluate tissue damage observed in experimental animal models of ARDS.

Keywords: Rat, ARDS, ALI, histopathology, score, lung

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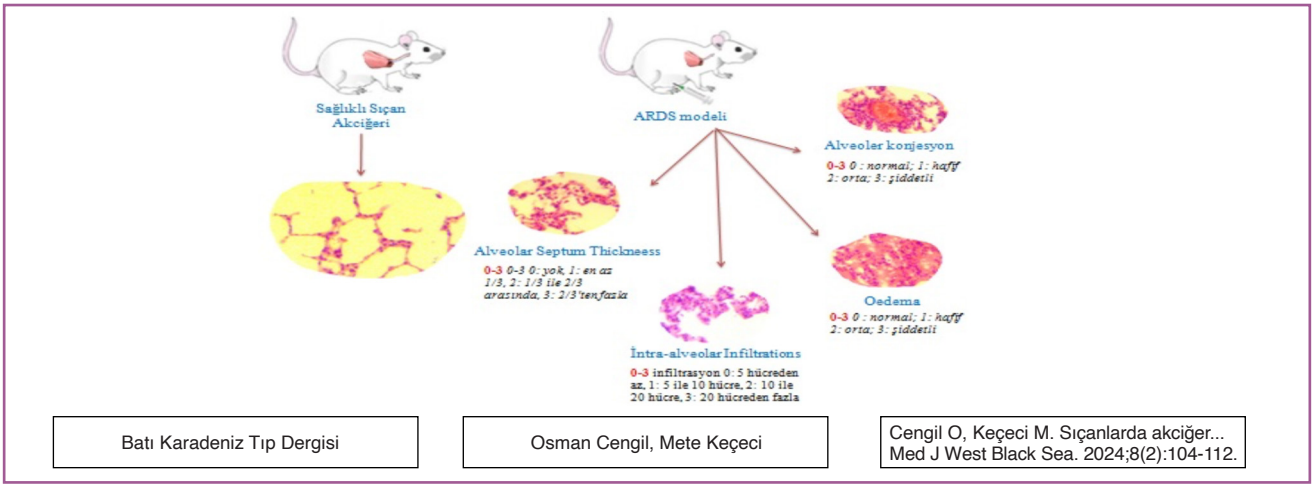
GRAFİKSEL ÖZET



ÖZ

Akut solunum sıkıntısı sendromu (ARDS), bakteriyel ve viral pnömoninin yanı sıra birçok nedenin tetiklediği, insanlarda klinik parametrelerin çok iyi tanımlanmış ciddi bir akciğer reaksiyonudur. Ancak deneysel hayvan modelinde ARDS parametrelerine ilişkin kesin bir tanımlama mevcut değildir. Amerikan Toraks Derneği 2010 çalıştay raporuyla laboratuvar hayvanlarında ARDS varlığını belirleyen parankimal dokuda değişiklikler, alveoler-kapiller bariyerin bütünlüğünün değişmesi, iltihaplanma ve anormal akciğer fonksiyonu gibi histopatolojik ana özellikler tanımlamıştır. Bu parametreleri anlamak ve gözlemsel patolojik verileri istatistiksel analiz ve gelişmiş kesinlik için yarı niceliksel veya niceliksel verilere dönüştürmek için doku lezyonlarının skorlanması yöntemi kullanılmaktadır. Ancak farklı hayvan türlerinin ve farklı ARDS deneysel modellerin olması bu skorlama yöntemlerinde karmaşa neden olmaktadır. Bundan dolayı PubMed veritabanında son beş yıl içinde yapılan araştırmalarda ARDS deneysel modeller için kullanılan histopatolojik lezyon skorlama sistemleri detaylı bir şekilde incelenmiştir. Bu makalenin amacı, ARDS'in deneysel hayvan modellerinde gözlemlenen doku hasarını değerlendirmek için kullanılan farklı parametreler ve skorlama sistemleri hakkında kapsamlı bir rehber sunmaktır.

Anahtar Sözcükler: Sıçan, ARDS, ALI, histopatoloji, skor, lung

INTRODUCTION

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a life-threatening, highly heterogeneous disease with a global mortality rate of 30% to 50% (1,2). The current coronavirus disease (COVID-19) pandemic caused the development of ARDS in 29-42% of cases, while death occurred in 15-52% of these cases (3,4). The causes of ARDS are many and varied. However, they can generally be divided into two main categories: direct damage to the lungs, such as lung infection, or indirect damage to the lungs, such as sepsis (5).

According to the etiology of ARDS, it is examined under two main headings: pulmonary ARDS (ARDSp) and extrapulmonary ARDS (ARDSexp). When the origin of ARDS is direct lung disease or damage, it is called ARDSp, while in cases where the lungs are affected by a systemic inflammatory response such as sepsis, the term ARDSexp is used. The underlying mechanism in both ARDSp and ARDSexp is diffuse alveolar and capillary endothelial damage due to disruption of the alveolar-capillary barrier. There are studies reporting differences in pathophysiological, morphological and respiratory mechanical features between the two (6). Although in vitro models such as cell cultures provide valuable information on ARDS, they remain inadequate due to the complex nature of the disease, necessitating animal models for preclinical studies. The choice between animal species for these models depends on the aspects of the disease to be mimicked and the specific treatment being evaluated (7).

In 1994, the American-European Consensus Conference published a wide range of definitions of ALI/ARDS created by clinicians and researchers (8). Subsequently, after the Berlin definition was proposed in 2012, the clinical diagnosis of ARDS was updated and consisted of four main features (9). These criteria are as follows: patients typically exhibit acute respiratory symptoms that worsen over time, accompanied by clear evidence of bilateral pulmonary oedema visible on chest X-ray or CT scan. It is crucial to note that this respiratory failure cannot be attributed to heart failure or fluid overload. Furthermore, the PaO_2/FiO_2 ratio must be

below 300, with no indication of elevated pulmonary artery pressure. The severity of ARDS is determined by the PaO_2/FiO_2 ratio and the level of positive end-expiratory pressure (PEEP) required for ventilation.

While it is possible to assess animal models according to the Berlin Criteria, the short duration of ARDS injury models, lack of equipment for procedures such as catheterisation, arterial blood gas, chest radiography and cardiac echocardiography, and the practical impossibility of many experimental methods make this approach impractical (10). Therefore, an alternative procedure is to use the histopathological criteria of ALI observed in humans (11). The pathological equivalent of ALI in humans is inflammatory infiltration, characterised by diffuse alveolar damage, alveolar wall thickening and hyaline membrane formation (12). While existing animal models of ALI do not completely mimic all pathological features in humans, semi-quantitative scoring of damage features by observers is the most common form of analysis used to evaluate histological samples (13).

Histopathology is the definitive method of diagnosing disease in living organisms. By examining a tissue sample under a microscope, morphological changes are evaluated in detail. In this way, the observer can describe the tissue morphology and compare the observed abnormalities with healthy tissue. Experts have established a simple scoring system that describes semi-quantitative grading of lesion severity (low, medium, high) to provide reproducible diagnoses and even as a reliable predictor of clinical effects. Determining the extent and severity of lesions is limited in traditional histopathology due to difficulties in the application of rational scales [1,2,3] and natural observer variability (14,15).

Lung Histology

The respiratory system is composed of two primary sections: the conductive and respiratory components. The conductive part, which includes the nose, nasopharynx, larynx, trachea, bronchi, and bronchioles, serves as the pathway for air to enter and leave the lungs. The respiratory part, located within the lungs, begins with the respiratory bronchiole and branches into alveolar ducts and sacs, culminating in the alveoli, where the vital gas exchange process of breathing takes place (16-18).

The conductive zone, contains specialised cells (ciliary, goblet, basal, brush, and neuroendocrine) that condition the inhaled air by warming, humidifying, and filtering it before it reaches the respiratory zone. The exchange of gases takes place in the respiratory zone. Alveoli, tiny air sacs, are the primary structures for this process, consisting of type I and II pneumocytes, brush cells, and alveolar macrophages. These structures, together with supporting tissue and a dense capillary network, form the alveolar walls, which also contain pores for inter-alveolar communication (16-18).

Increased Injury as Evaluated by a Standard Histological-Score

In 2010, the American Thoracic Society (ATS) designed a semi-quantitative histological grading system for the assessment of the severity of lung injury in ARDS. This standardized method involves examination of at least 20 microscopic fields under a 40x objective, enabling a detailed assement by trained pathologists. By providing a clear framework for scoring lung damage, this system facilitates both individual assessments and objective comparisons across multiple animal models, allowing for robust statistical analysis of histological findings (10).

Due to the inconsistent distribution of histological changes in many experimental animal models of ARDS, a comprehensive evaluation of lung tissue is essential to accurately document and grade pathological lesions. Therefore, whenever feasible, the entire lung should be examined to obtain a complete picture of the disease process.

To facilitate statistical analysis, lesions can be score using both quantitative and semi-quantitative methods. Semi-quantitative approaches assign numerical grades to observed lesions, while quantitative methods involve the measurement of specific lesion characteristics. These techniques improve the understanding of the distribution and severity of inflammation by providing numerical data.

Semi-quantitative scoring systems are often used to assess stained tissues and provide a preliminary step in the statistical comparison of treatment groups. These systems involve assigning numerical grades or scores to tissue changes to facilitate subsequent statistical analysis. While various semi-quantitative approaches exist, the optimal method depends on the study design and research questions. Common scoring systems include binary (affected or unaffected), graded ranking, and ordinal scales (19).

Scoring, often referred to as grading, is a valuable method of extracting data from biological systems, such as tissues, to enable analysis and group comparisons. This technique can be applied at various stages of examination, including pre-mortem imaging, post-mortem macroscopic observation, and microscopic histological analysis.

To determine the convenient histological scoring system for any tissue, basic principles such as Lesion parameters and Scoring definitions must be taken into account. Although lesion parameters are defined in detail in the ATS report, different lesion definitions have been made in the literature or more prominent lesion parameters have been used due to the wide variety of etiologies of ARDS. Scoring definition is divided into categories, and it is useful to have clear language that both characterizes and sets boundaries for each category. Interval and ordinal scoring systems are mostly used. Interval scoring involves measuring sample

quantities on a scale between two defined endpoints, with an arbitrarily assigned zero value. This method allows for comparisons between samples based on the differences in their assigned values. Ordinal scoring uses specific terms such as “normal,” “mild,” “moderate,” or “severe,” or “0,” “1,” “2,” and “3,” according to a category that shows a sequential progression in severity (19).

ALI was defined as the evaluation of six different variables (neutrophils in the alveolar space and/or in the interstitial space, proteaceous debris filling the airspaces, presence of hyaline membranes, alveolar septal thickening, and alveolar congestion) as shown in Table 1 in the studies examined in general (20). In addition, microscopic images of these six variables are included in Figure 1.

Table 1: Histologic lung injury parametre and result (20).

Parameters	Score per field		
	1	2	3
1. Neutrophils in the alveolar space	None	1-5	>5
2. Neutrophils in the interstitial space	None	1-5	>5
3. Hyaline membranes	None	1	>1
4. Proteinaceous ebris filling the airspaces	None	1	>1
5. Alveolar septal thickening	<2x	2x-4x	>4x
6. Alveolar congestion	None	1-5	>5

The presence of neutrophils in the alveolar or the interstitial space: Neutrophils are the most ample type of white blood cells and play a vital role in the body’s immune system, fighting off invading pathogens and infections. Normally, the alveolar space is sterile and devoid of neutrophils, but a small number of neutrophils are present in the interstitial space. In inflammatory or infectious conditions, neutrophil accumulation in both alveolar and interstitial spaces significantly increases, indicating a potential pathological process (21,22).

Presence of hyaline membranes: These are non-cellular, protein-rich deposits formed on the alveolar wall (23).

Formation of proteinaceous debris in the alveolar space: ARDS disrupts the delicate air-blood barrier, leakage of protein-rich fluid into the alveoli. This accumulation of proteinaceous debris, visible on histological examination as a slightly eosinophilic material, is a hallmark of the disease. Its appearance can vary from homogeneous to fibrous, reflecting the severity of the lung injury. Methods such as measuring lung wet-to-dry weight ratios are used to quantify pulmonary edema associated with this protein-rich leakage (24).

Alveolar septa thickening: Accurate assessment of alveolar wall thickness for reliable histological examination requires a thorough evaluation of the entire lung sample,

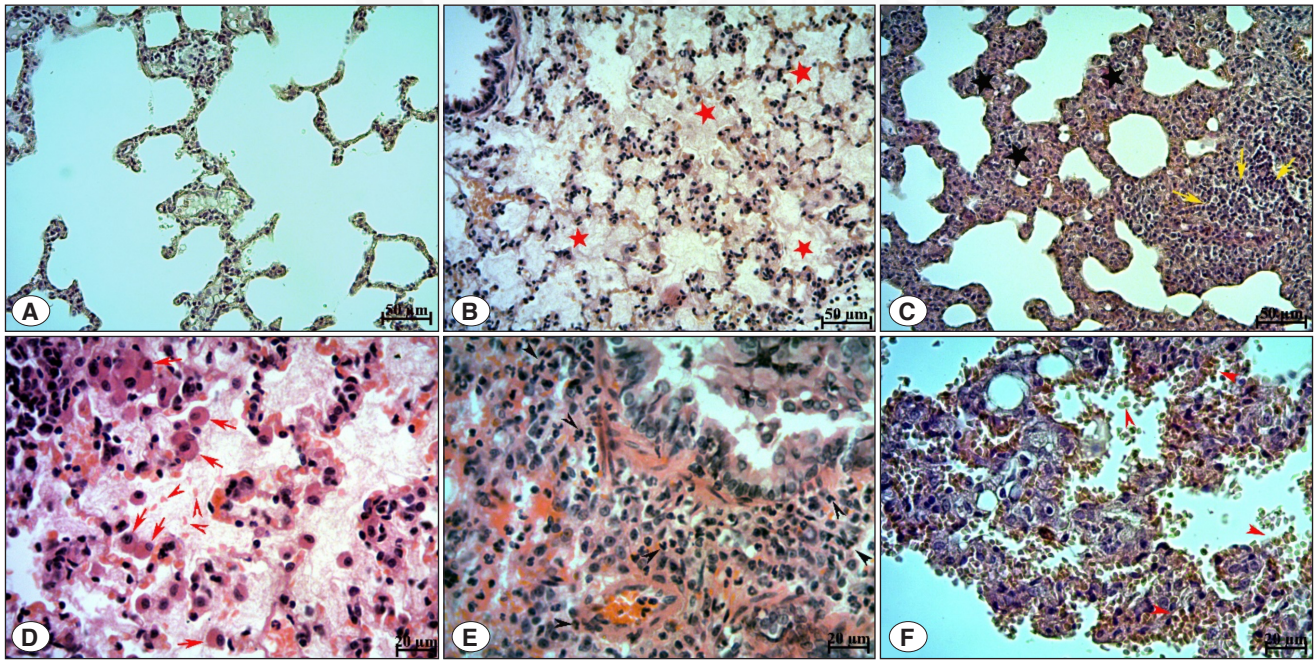


Figure 1: A) Normal lung tissue, **B)** Intraalveolar edema (red star) in α-Naphthylthiourea (ANTU) model, **C)** Alveolar septum thickening (black star) and lymphocytic infiltration (yellow arrow) in the CCl4 model, **D)** Alveolar and septal macrophages (red star) in the ANTU model, **E)** Neutrophilic infiltration (black arrowhead) in the lipopolysaccharide model, **F)** Alveolar and septal hemorrhage (red arrowhead) in the CCl4 model. Scale bar; A, B, C: 50 µm, D, E, F: 20 µm. H&E staining. (Images provided by Osman Cengil’s archive).

especially when comparing different animals or groups. To avoid over-interpretation, septa that are more than twice as thick as normal-appearing septa in control animals should be accepted important for this criterion. (25).

Alveolar congestion: Filling of alveoli and alveolar capillaries with erythrocytes (26).

MATERIAL and METHODS

SELECTION OF SCORING SYSTEMS

To identify relevant scoring systems, a comprehensive search was conducted on PubMed (https://pubmed.ncbi.nlm.nih.gov/) within the last five years. The search strategy combined terms like "rat," "ALI," "score," "histopathology," "lung," "alpha-naphthylthiurea," "lipopolysaccharide," and "acid aspiration.". This led to the identification of 165 publications as of 20 December 2023. Full-text versions of all identified publications were retrieved and carefully reviewed to extract and define multiparametric, semi-quantitative scoring systems specifically designed for histopathological assessment in rat models.

In the literature, histological scoring of the lung uses the area of leukocyte infiltration in the interstitial tissue as a percentage by separating four sections. It is scored as 0%=0; 25%=1; 50%=2; 75%=3 and; more than 75%=4 (27). The presence of leukocytes on the alveolar surface is scored according to the amount and 0=no cells; 1=few cells; 2=high amount of cells; 3=full of cells; 4=full of outspread leukocytes. The content of alveolar exudate containing edema fluid, cellulose, hyaline membrane and meconium is scored according to the total amount in the image. 0=no exudate content; 1=low exudate content; 2=significant exudate content; 3=filled with exudate content; It is scored as 4= completely filled with exudate content (28).

Other studies in the literature examined five independent variables in lung histological scoring, including neutrophils in the alveolar (A) and interstitial space (B), hyaline membranes (C), proteinaceous residues filling the airspaces (D) and alveolar septal thickening (E) and used a ranked score from 0 to 2 according to the interest attributed to each feature. For the amount of neutrophils, 0: none, 1: 1-5 cells, 2: more than 5 cells; for hyaline membranes and proteinaceous residues filling the air spaces, definitions were made as 0:none, 1:1 membran and residu, 2:more than 1 membran and residu and Alveolar septal thickening 0: 2× thinner, 1:2×-4× thickness, 2: 4× thicker. Score = [(20 x A) + (14 x B) + (7 x C) + (7 x D) + (7 x D) + (2 x E)]/(number of fields x 100). These values were summed and then normalized by the number of fields examined. The resulting injury score was a continuous value ranging from zero to one, providing a quantitative measure of lung injury severity (10,29-32).

In other literature reports, a 5-point (0-4) scoring system was used, including parameters for alveolar congestion, haemorrhage, infiltration or aggregation of neutrophils in the air space or vascular wall, and thickness of the alveolar wall / formation of the hyaline membrane. 0: minimum damage; 1: mild damage; 2: medium damage; 3: severe damage; and 4: maximum damage (33-44).

Other studies in the literature used a 4-point (0-3) scoring system including the following parameters: edema, neutrophil margination and infiltration of the tissue, hyperaemia and congestion, intra-alveolar haemorrhage, intra-alveolar debris and cellular hyperplasia formation. 0: normal; 1: mild effect; 2: medium presence of this feature; and 3: severe effect (45,46).

Karakişi et al. used a 4-point (0-3) scoring system including infiltration, thickness of the alveolar septum, hyaline membrane and accumulation of alveolar debris parameters. In this scoring system, 0: none, 1: less than 5%, 2: less than 25% and 3: less than 50% were defined for the first 3 parameters, while Matute-Bello (10) definition was used for alveolar septum thickness (47).

Lin et al. used a 4-point (0-3) score system including alveolar collapse and alveolar septum thickness parameters. 0 = no parameters detected, 1: presence of parameters in less than 15% area, 2: presence of parameters in 15%-25% area, 3: presence of parameters in 25%-50% area, 4: presence of parameters in 50%-75% area and 5: presence of parameters in more than 75% area per HPF (100x) (48).

Ren et al. graded the degree of alveolar structure injury on a scale of 0 to 7 and scored the pulmonary interstitial, edema, and alveolar hemorrhage parameters. 0: non-pathological alterations in the lung; 1: lung parameters less than 25%; 2: lung parameters less than 25% and few neutrophils in the interstitial tissue; 3: lung parameters 25-50%; 4: lung parameters 25-50% and many neutrophils in the interstitial tissue and alveoli; 5 lung parameters 50-75%; 6: lung parameters more than 75%; 7: lung parameters more than 75% and neutrophils in all of the interstitial tissue and alveoli (49).

Yu and Li defined scoring on a scale from 0 (normal) to 5 (maximum) including parameters such as thickening of alveolar walls and epithelium and infiltration cell numbers (50).

Mu et al. used a 4-point (0-3) scoring system including alveolar septae, alveolar haemorrhage, intra-alveolar fibrin and infiltrations. In this scoring system, for alveolar septae and intra-alveolar fibrin 0: none, 1: at least 1/3, 2: between 1/3 and 2/3, 3: more than 2/3; alveolar haemorrhage 0: none, 1: 1 to 5 erythrocytes 2: 5 to 10 erythrocytes, 3: more than 10 erythrocytes; intra-alveolar infiltrations 0:> 5 cells, 1: 5-10 cells, 2: 10-20 cells, 3: more than 20 cells (51).

Al-Gabri et al. defined mild, severe and very severe scoring for perivascular edema, endotheliosis, inflammatory cell adhesions, muscular wall proliferations, vacuolar media and perivascular fibrosis parameters (52). Wu et al. defined a grading scale of 0-5 according to the presence of lesions and scored, edema, fibrin and hemorrhage parameters: 0: no parameters; 1: subpleural parameters; 2: interlobular parameters; 3: alveolar parameters; 4: alveolar septal congestion; and 5: in the hyaline membrane of the septa of the alveoli (53).

Lung score range and parameters are given in Table 2.

Table 2: Lung injury range and scoring

Ranges	Parameters	Score per field
%	Area of leukocyte infiltration in the interstitial tissue	0%=0; 25%=1; 50%=2; 75%=3 and; more than 75%=4 (27)
0-2	amount of neutrophils	0: none, 1: 1-5 cells, 2: more than 5 cells (10, 29-32)
	Hyaline membrane, edema	0:none, 1:1 membran and residu, 2:more than 1 membran and residu (10, 29-32)
	Alveolar septal thickening	0: 2× thinner, 1:2×-4× thickness, 2: 4× thicker (10, 29-32,47)
0-3	Edema, neutrophil margination and infiltration of the tissue, hyperaemia and congestion, intra-alveolar haemorrhage, intra-alveolar debris and cellular hyperplasia formation	0: normal; 1: mild effect; 2: medium presence of this feature; and 3: severe effect (45,46).
	İnfiltration, hyaline membrane, alveolar debris accumulation	0: none, 1: less than 5%, 2: less than 25% and 3: less than 50% (47)
	Alveolar collapse and alveolar septum thickness	0 = no parameters detected, 1: presence of parameters in less than 15% area, 2: presence of parameters in 15%-25% area, 3: presence of parameters in 25%-50% area, 4: presence of parameters in 50%-75% area and 5: presence of parameters in more than 75% area per HPF (100x).
	Alveolar septae, intra-alveolar fibrin	0: none, 1: at least 1/3, 2: between 1/3 and 2/3, 3: more than 2/3 (51)
	Alveolar haemorrhage	0: none, 1: 1 to 5 erythrocytes 2: 5 to 10 erythrocytes, 3: more than 10 erythrocytes (51)
	Intra-alveolar infiltrations	infiltrations 0:> 5 cells, 1: 5-10 cells, 2: 10-20 cells, 3: more than 20 cells (51)
0-4	Total number of leukocytes on the alveolar surface	0=no cells; 1=few cells; 2=high amount of cells; 3=full of cells; 4=full of outspread leukocytes (28)
	Total content of alveolar exudate including edema fluid, cellulose, hyaline membrane and meconium	0=no exudate content; 1=low exudate content; 2=significant exudate content; 3=filled with exudate content; 4= completely filled with exudate content (28)
	Alveolar congestion, haemorrhage, infiltration or aggregation of neutrophils in the air space or vascular wall, and thickness of the alveolar wall / formation of the hyaline membrane	0: minimum damage; 1: mild damage; 2: medium damage; 3: severe damage; and 4: maximum damage (33-44).
0-5	Thickening of alveolar walls and epithelium and infiltration cell numbers	defined scoring on a scale from 0 (normal) to 5 (maximum) (50)
	Alveolar structure damage	edema, fibrin and hemorrhage parameters: 0: no parameters; 1: subpleural parameters; 2: interlobular parameters; 3: alveolar parameters; 4: alveolar septal congestion; and 5: in the hyaline membrane of the septa of the alveoli (53)
0-7	Alveolar structure damage	pulmonary interstitial, edema, and alveolar hemorrhage parameters. 0: non-pathological alterations in the lung; 1: lung parameters less than 25%; 2: lung parameters less than 25% and few neutrophils in the interstitial tissue; 3: lung parameters 25-50%; 4: lung parameters 25-50% and many neutrophils in the interstitial tissue and alveoli; 5 lung parameters 50-75%; 6: lung parameters more than 75%; 7: lung parameters more than 75% and neutrophils in all of the interstitial tissue and alveoli (49).

DISCUSSION

After an extensive search of the literature, 35 semi-quantitative, multi-parameter scoring systems originally designed for histopathology of rat models were identified.

The primary purpose of scoring systems is to assess the impact of experimental interventions on microscopic tissue structure. Consequently, the parameters included in these systems should be carefully selected to capture potential morphological changes. Surprisingly, a consistent lack of explicit rationale for parameter selection was observed during the literature review. Although the selection of parameters used in scoring systems is usually based on a general knowledge of the pathological processes of the disease studied in animal models, detailed explanations or discussions of how this parameter selection is made are unfortunately not very common. This leads to problems such as the diversity of parameters used in different studies and the lack of standardisation.

A 0-3 and 0-4 scoring system was used in two separate studies in which the authors of this review were involved, a postgraduate thesis study evaluating the effects of carbon tetrachloride in rat lung tissue and a simulation of ANTU-mediated ALI in rat lung tissue. This scoring methodology was deemed appropriate for the specific parameters examined in both studies. However, edema dominance was evident in the ANTU model, edema was not observed in the CCl4 model (54,55). This result clearly demonstrates the importance of the lung injury model in the choice of scoring criteria.

CONCLUSION

This study did not critically evaluate the quality or effectiveness of the scoring systems presented. The suitability of a particular scoring system depends on the specific research question, hypothesis, animal model and disease pathogenesis. Nevertheless, this review provides a comprehensive overview of existing systems to facilitate a more informed choice for future research endeavours.

Before determining the scoring system to be used, all experimental groups should be examined and lung injury parameters appropriate to the experiment should be determined. Once these parameters have been established, the lesions in all groups should be examined and a grading system appropriate to the pathology established. These parameters and the scoring system should be recorded in detail. This grading system appropriate to the experiment should be applied to all groups independently by at least two histologists.

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Author Contributions

Osman Cengil designed the study. **Mete Keçeci, Osman Cengil** contributed to the literature review and writing of the article.

Conflicts of Interest

Authors have no relevant financial or non-financial interests.

Financial Support

None.

Ethical Approval

Since it is not an experimental or human study and is a review, ethical approval is not required.

Review Process

Extremely and externally peer-reviewed.

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