**Name of journal: World Journal of Neurology**

**ESPS Manuscript NO: 4382**

**Columns: MINIREVIEWS**

**Variation in experimental autoimmune encephalomyelitis scores in a mouse model of multiple sclerosis**

Takeuchi C *et al.* EAE scores in a mouse model of MS

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**Author contributions**: Takeuchi C wrote the manuscript and generated the tables; Yamagata K contributed to the writing of the manuscript; Takemiya T contributed to the study design and wrote the manuscript.

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**Received:** June 28, 2013  **Revised:** July 31, 2013

**Accepted:** August 16, 2013

**Published online:**

**Abstract**

Multiple sclerosis (MS) is a common demyelinating central nervous system disease associated with progressive physical impairment. To study the mechanism underlying disease pathogenesis and develop potential treatments, experimental autoimmune encephalomyelitis (EAE) is often used as an animal model. EAE can be induced in various species by introducing specific antigens, which ultimately result in motor dysfunction. Although the severity of the paralysis is indicated using the EAE score, there is no standard scoring system for EAE signs, and there is significant variability between research groups with regard to the exact EAE scoring system utilized. Here, we describe the criteria used for EAE scoring systems in various laboratories and suggest combining EAE score with another quantitative index to evaluate paralysis, such as the traveled distance, with the goal of facilitating the study of the mechanisms and treatment of MS.

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**Key words:** Multiple sclerosis; Experimental autoimmune encephalomyelitis; Experimental autoimmune encephalomyelitis score; Motor dysfunction

**Core tip:** Multiple sclerosis (MS) is a common demyelinating central nervous system disease associated with progressive physical impairment. Experimental autoimmune encephalomyelitis (EAE) is often used as an animal model to study MS. EAE can be induced in various species by introducing specific antigens, and the severity of the paralysis is indicated using the EAE score. The score is simple and easy to use however, its application varies between laboratories, and the scoring is dependent on the subjective bias of the researchers. We described the criteria used for the EAE scoring systems in various laboratories, to facilitate the study of MS.

Takeuchi C, Yamagata K, Takemiya T. Variation in EAE scores in a mouse model of multiple sclerosis. *World J Neurol* 2013;

**Available from:** URL: http://www.wjgnet.com/2218-6212/

**DOI:** http://dx.doi.org/10.5316/wjn.

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the brain and spinal cord. More than 100 years have passed since the clinical and pathological characteristics of MS were first described in the medical literature[1]. Despite extensive research since then, the pathogenesis underlying MS is still not fully understood. There are more than 2500000 patients with MS worldwide, and the prevalence is approximately 4 to 150 per 100000 in the population[2,3]. The incidence of MS varies across the world; it is quite high in northern Europe but lower in Asian and African countries[3,4]. MS typically manifests in young adulthood, primarily between the late twenties and early forties. Although the clinical course and prognosis of the disease demonstrate individual differences, 50% of patients need help walking, or in some cases require a wheelchair within 15 years of the initial disease onset[4]. The demyelinating lesion of MS has been described as “disseminated in time and space”[5]. The clinical course of the disease is characterized by four major subtypes: relapse-remitting, secondary progressive, primary progressive, and progressive-relapsing MS[1,4,6]. Approximately 80% of all patients initially manifest with relapsing-remitting type MS. Symptoms and signs typically become aggravated over a period of several days, and the condition then gradually stabilizes. Patients often improve spontaneously or in response to treatment within weeks. Eventually, approximately 65% of patients with relapsing-remitting MS enter the secondary progressive phase[4]. Moreover, in 20% of all patients, the illness gradually worsens after onset, which is defined as primary progressive MS[1,4]. There is no consensus definition in progressive-relapsing MS[6].

Because the opportunity to obtain central nervous system tissue from individual patients is rare, animal models of MS have been developed to investigate the pathogenesis and treatment of the disease. Experimental autoimmune encephalomyelitis (EAE) is the most popular animal model of MS[7-11]. EAE is characterized by inflammatory infiltrates consisting of T-lymphocytes, B-lymphocytes, macrophages, and focal demyelinating plaques in the CNS; these features are also observed in MS. EAE is induced in various species, including rodents and primates, either by active immunization using a myelin antigen in adjuvant (active EAE) or by the adoptive transfer of encephalitogenic T cells (passive EAE)[10,11]. In addition, T cell receptor transgenic mice have been generated as a spontaneous EAE model. The characteristics of these mice are variable, and thus, most of the spontaneous EAE mice are also defined as atypical EAE[8,12,13]. Commonly used murine EAE models manifest motor dysfunction as ascending flaccid paralysis, beginning with a flaccid or limp tail[7,10,11]. The paralysis progresses from the hind limbs to the fore limbs and is occasionally followed by urinary incontinence and fecal impaction (classical EAE models)[7,10]. Lesions are predominantly localized to the spinal cord in classical EAE. The standard EAE mouse model is induced using myelin proteolipid protein (PLP) peptide (amino acids 139-151), which causes relapse-remitting EAE in SJL mice. In addition, the myelin oligodendrocyte glycoprotein (MOG) peptide (amino acids 35-55) causes monophasic EAE with an incomplete recovery in C57BL/6 mice[8]. Several reports have demonstrated real primary progressive or secondary progressive EAE, in which mice die as a result of disease progression[14]. However, variations from the classical EAE phenotype, such as ataxia or the head rolling phenomenon rather than limb paralysis, have been described and are referred to as atypical EAE[15-17]. The clinical signs observed in atypical EAE models reflect an increase in inflammation in the brain compared to classical EAE models. The characteristics of spontaneous EAE mice are also defined as atypical EAE. In the active EAE model, the mice are immunized by subcutaneous injection of the myelin antigen with complete Freund’s adjuvant (that is, the antigen is emulsified in paraffin oil containing inactive *Mycobacterium tuberculosis*). Intravenous or intraperitoneal injection of pertussis toxin is required to increase the incidence of EAE induction. Although the signs of motor dysfunction depend on the type of EAE model, paralysis usually begins within 9 to 14 d after sensitization[10]. Passive induction of EAE in naïve mice is achieved by the adoptive transfer of T cells isolated from active EAE mice that have been primed with myelin antigens. The day of onset of visible EAE signs varies, and depends on the model; however, signs usually appear between 10 and 15 d after induction[11].

In this review, unless otherwise noted, we describe the development of the signs of classical EAE and the evaluating system used by researchers. Many researchers misuse terms for evaluating EAE signs; for example, “EAE symptom” or “clinical assessment of EAE.” The term “symptom” and “clinical” should only be used in the context of humans; and thus, the term “signs” must be used instead of “symptom.” In addition, “clinical” must not be used in EAE studies. The severity of EAE is generally evaluated using an EAE score (occasionally referred to as the EAE scale or grade). Mice are scored daily after the day of sensitization to precisely detect the time of disease onset and to investigate the progression of EAE. The commonly used EAE scores are 0 to 5 or 0 to 6 point scales (Table 1-3)[10,18-29]; however, there are problems using this method. First, each laboratory has its own method for evaluating the severity of EAE; these methods have not been standardized between laboratories. In most laboratories, a loss of tail tone is recognized as a score of 1, which is designated as a “loss of tail tonicity”[18,20,23,26,29], “flaccid tail”[19,22], “limp tail”[25,28], “tail weakness”[24] and “tail atony”[27]. In particular, a complete loss of tone has been required in previous studies[25-29]. Incontrast, Sobel *et al*[21] described a score of 1 as “decreased tail tone or slightly clumsy gait”. A score of 2 is identified by symptoms of paralysis/weakness of the hind limbs, impairment of the righting reflex (the mice have difficulty turning over after being laid down on their back, but there are no observed locomotor difficulties), tail paralysis and gait disturbance. The term “paralysis” indicates a complete or partial loss of voluntary movement. The prefix “para” means “both” and “plegia” means severe weakness. The term “paraplegia” is defined as a severe symmetrical muscle weakness of both lower limbs. “Paraparesis” commonly means slight or partial paralysis of both lower limbs; however, the definition of these terms is subjective and indistinct. These symptoms have been described as “mild hind limb or unilateral paralysis”[19,22,26,28] or “hind limb weakness”[18,27], “impaired or poor righting reflex”[20,21,29] or “loss of the righting reflex”[24], “tail atony or paralysis”[21,24] or “flaccid tail”[23], and “moderately clumsy gait”[20] or “abnormal gait”[28]. Most laboratories define a score of 3 as hind limb paralysis[18-20,23,25,27-29] or weakness[21,22]; however, Pollak *et al*[24]. included “loss of the righting reflex”. Thus, the same EAE score may indicate different conditions in the EAE mice. Furthermore, the score is not a quantitative analysis. Some researchers use "in-between" scores (0.5 point) when the symptom lies between the two defined scores (Table 3). Tsunoda *et al*[26] and Storch *et al*[27]. only showed an in-between score of between 0 and 1 (0.5 score) but not between other intervals. In contrast, there were in-between scores for all intervals except for a score of 4.5 in a method used by Greter *et al*[28]. Most researchers judge the statistical significance of EAE signs by comparing the scores of two groups of EAE mice: *i.e.*, wild type EAE mice and genetically modified EAE mice, or treated EAE mice and non-treated EAE mice. Furthermore, the method of assessing an EAE score depends on subjective observation. For example, researchers hold the base of the mouse tail to judge tail limpness. In addition, they touch or perform a toe pinch to evaluate the gait condition of the mice [10,11]. Finally, designation of the sign as “mild” or “severe” is ill-defined; thus, we need to eliminate the observer bias in the judgment of EAE scores. In addition, the cumulative score is obtained by the sum of the daily EAE score using previously described methods.

Not only is a standard behavior scoring system for EAE needed, but a different method for evaluating EAE progression is also required. After sensitization, in general, the mice are weighed regularly and scored for EAE signs. Body weight loss is a common feature of EAE that usually precedes paralysis, and low body weight remains during the recovery phase[12,30,31]. Body weight begins to increase during the chronic phase of the disease; thus, weight loss is an important sign during the acute stage of EAE. Jones *et al*[32] observed the relationship between the EAE score and rotarod performance, the grip strength test of both the fore limbs and hind limbs, and the open field test. In the open field test, gait and rearing events of natural exploratory behavior could be detected. A drawback of this method is that severe (and, in some cases, moderate) paralysis prevents animals from performing the rotarod and grip strength tests. The Basso, Beattie, and Bresnahan (BBB) score is a well-established technique for evaluating spinal cord injury in animal models[33,34]; thus, this score may also be used in EAE studies[35,36]. To determine the BBB score, mice are placed in an open field area with two observing researchers. The BBB sub-components include limb movement, trunk position, paw placement, walking, predominant paw position, trunk instability and tail position. BBB is rated on a scale from 0 (no observable hind limb movements) to 21 (consistent coordinated gait, consistent toe clearance, predominant paw position is parallel at initial contact and lift-off, tail consistently up, and consistent trunk stability), which represents the sequential recovery of spinal cord injury[33]. Kerschensteiner reported that the BBB score is predictive of the site and extent of the pathological lesion and is more sensitive for assessing the development of EAE than are EAE scores. However, the BBB score must be determined in precisely 21 stages by two proficient observers[36]. Thus, a simple, universal and clear-cut method is needed for evaluating motor dysfunction in EAE. Our recent study suggests that the traveled distance is a sensitive and accurate marker of motor dysfunction in a MS mouse model (unpublished).

In conclusion, the EAE score is simple and easy to use; however, its application varies between laboratories, and scoring is dependent on the subjective bias of the researchers. To achieve the standard scoring system in EAE, it is necessary to define the terms for signs and to clarify the criteria for the signs in the EAE score. Furthermore, ambiguous representation, *e.g*., weak or strong weakness of the hind limb, must be eliminated. We suggest that a standardized EAE scoring system should be implemented and combined with another quantitative index, such as the distance traveled in the open field test, which would provide a substantial advantage over the current conventional EAE scoring methods.

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**P-Reviewers** Tsunoda I, Haueisen J **S-Editor** Song XX **L-Editor E-Editor**

**Table 1 Commonly used classical experimental autoimmune encephalomyelitis score**

|  |  |  |
| --- | --- | --- |
| Score | EAE signs | Observation |
| 0 | No signs of EAE | Hind legs are extended; tail extends up and moves; tail wraps around cylindrical object; normal gait |
| 0.5 | Partially limp tail | Hind legs are extended; tip of tail droops and/or does not wrap around cylindrical object; normal gait |
| 1 | Paralyzed tail | Hind legs are extended; tail droops and does not wrap around cylindrical object; normal gait |
| 2 | Loss in coordinated movement; hind limb paralysis | Hind legs contract when held at the base of tail; mouse walks with uncoordinated movement; hind limbs reflex when toes are pinched; limp tail |
| 2.5 | One hind limb paralysis | Mouse drags one hind limb; one hind limb does not respond to pinch; limp tail |
| 3 | Both hind limbs paralysis | Mouse drags both hind limbs; both hind limbs do not respond to toe pinch; limp tail |
| 3.5 | Hind limb paralysis; weakness in forelimbs | Mouse drags hind limbs but has difficulty using forelimbs to pull body; forelimbs respond to toe pinch; limp tail |
| 4 | Forelimbs paralysis | Mouse cannot move; forelimbs do not respond to toe pinch; limp tail |
| 5 | Moribund | No movement; cold to the touch; altered breathing |

Note there are no scores denoted 1.5 and 4.5. The data available from reference 10. EAE: Experimental autoimmune encephalomyelitis.

**Table 2 Differences in experimental autoimmune encephalomyelitis scores among researchers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Swanborg[18] | Matstumoto *et al*[19] | Yamamura *et al*[20] | Sobel *et al*[21] |
| Strain | Lewis rat | Lewis rat | C57BL/6J mice | SJL/J mice |
| Antigen | MBP | MBP | MOG35-55 | PLP131-151 |
| 0 | ND | ND | No clinical signs | No disease |
| 1 | Loss of tail tonicity | Flaccid tail | Loss of tail tonicity | Decreased tail tone or slightly clumsy gait |
| 2 | Definite hind quarter weakness | Mild paraparesis | Impaired righting reflex | Tail atony and/or moderately clumsy gait and/or poor righting ability |
| 3 | Hind led paralysis | Severe paraparesis | Partial hindlimb paralysis | Limb weakness |
| 4 | ND | Moribund condition | Total hindlimb paralysis | Limb paralysis |
| 5 | ND | ND | ND | Moribund |
| 6 | ND | ND | ND | ND |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Kalyvas *et al* [22] | Mendel *et al*[23] | Pollak *et al*[24] | Takeuchi *et al*[25] |
| Strain | C57BL/6J mice | C57BL/6J mice  | SLJ/J mice | C57BL/6J mice |
| Antigen | MOG35-55 | MOG | PLP131-151 | MOG35-55 |
| 0 | Normal | No clinical signs | No neurological signs | No detectable signs of paralysis |
| 1 | Flaccid tail | Loss of tail tonicity | Tail weakness | Completely limp tail |
| 2 | Mild hindlimb paralysis | Flaccid tail | Tail paralysis | Loss of the righting reflex |
| 3 | Severe hindlimb weakness | Hind leg paralysis | Loss of righting reflex | Partial hind limb paralysis |
| 4 | Hindlimb paralysis  | Hind leg paralysis with hind body paresis | Hind limb paresis/paralysis  | Complete hind limb paralysis |
| 5 | Hindlimb paralysis and forelimb weakness or moribund | Hind and fore leg paralysis | Quadriplegia (immobility) | Total paralysis of allfour limbs |
| 6 | ND | Death | Death | Death |

ND: Not defined.

**Table 3 experimental autoimmune encephalomyelitis scores using the 0.5 point scale**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Tsunoda *et al*[26] | Storch *et al*[27] | Greter *et al*[28] | Kihara *et al*[29] |
| Strain | SJL/J mice | BN and DA rat | C57/BL mice  | C57/BL mice |
| Antigen | PLP | MOG1-125 | MOG35-55 | MOG35-55 |
| 0 | No clinical disease | ND | No detectable signs of EAE | No signs |
| 0.5 | Loss of tonicity of the distal half of the tail | Partial loss of tail tone | Distal limp tail | Mild loss of tail tone |
| 1 | Complete loss of tail tonicity | Complete tail atony | Complete limp tail  | Complete loss of tail tone |
| 1.5 | ND | ND | Limp tail and distal limb weakness | Mildly impaired righting reflex |
| 2 | Mild hind leg paresis | Hind limb weakness | Unilateral partial limb paralysis | Abnormal gait and/or impaired righting reflex |
| 2.5 | ND | ND | Bilateral partial hind limb paralysis | Hind limb paresis |
| 3 | Moderate hind leg paralysis | Hind limb paralysis | Complete bilateral hind limb paralysis  | Hind limbs paralysis |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 3.5 | ND | ND | Complete bilateral hind limb paralysis and unilateral forelimb paralysis | Hind limb paralysis with hind body paresis |
| 4 | Complete paraplegia | Tetraplegy, moribund state | Total paralysis of fore and hind limbs  | Hind and fore limb paralysis |
| 4.5 | ND | ND | ND | Moribund |
| 5 | quadriplegia, moribund state or death | Death | Death | Death |
| 5.5 | ND | ND | ND | ND |
| 6 | ND | ND | Death | ND |

ND: Not defined.