Overview

Identifying and Implementing Endpoints for Geriatric Mice

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The types of changes in physical appearance and behavior that occur in elderly people similarly develop in elderly animals. Signs and symptoms that might cause concern in younger people or mice may be normal in their elderly but generally healthy counterparts. Although numerous scoring methods have been developed to assess rodent health, these systems were often designed for young adults used in specific types of research, such as cancer or neurologic studies, and therefore may be suboptimal for assessing aging rodents. Approaches known as frailty assessments provide a global evaluation of the health of aged mice, rats, and people, and mouse frailty scores correlate well with the likelihood of death. Complementing frailty assessment, prediction of imminent death in aged mice can often be accomplished by focusing on 2 objective parameters—body weight and temperature. Before they die, many (but not all) mice develop marked reductions in body weight and temperature, thus providing signs that close monitoring, intervention, or preemptive euthanasia may be necessary. Timely preemptive euthanasia allows antemortem collection of data and samples that would be lost if spontaneous death occurred; preemptive euthanasia also limits terminal suffering. These approaches to monitoring declining health and predicting death in elderly research mice can aid in establishing and implementing timely interventions that both benefit the research and reduce antemortem suffering.

Abbreviations: CFI, clinical frailty index; FI, frailty index; PFI, physiological frailty index; TxBW, product of temperature and body weight

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Endpoints: An Overview

At least 2 considerations are essential to selecting appropriate experimental endpoints for any given experiment or animal species. First, the scientific purpose of the experiment must be clearly understood, clearly stated, and well justified, with precise explanation of exactly what the study is intended to accomplish. Furthermore, the research plan should precisely and clearly state exactly what data are necessary to achieve the scientific goal. Many studies collect many types of data from individual animals. Although this approach maximizes the information that can be gained from each study and each animal, it might obscure the key data that are necessary to answer the main experimental question. Essential data and corroborative data must be delineated to discern when the minimal goals of the scientific study have been achieved. For example, in studies of cancer development or treatment, the goals may be attained and the study ended for all or individual subjects after a specific time interval elapses subsequent to tumor implantation or drug administration, or, alternatively, if or when a tumor mass of a given minimal size is detected by palpation or *in vivo* imaging. Everyone involved in the study should understand exactly when and how the scientific goals of the study will be achieved.

The second key feature for selecting the ideal endpoint is the avoidance or minimization of animal harm. However, 2

considerations complicate this effort. First, the condition under investigation may itself cause pain or discomfort that perhaps cannot be alleviated completely even in human patients (for examples, see references 4, 11, and 35). Second, analgesic medications and unalleviated pain both have the potential to modify data and can thereby create different interpretational confounds and compromise reproducibility.^{14,38} Because of the potential for animal harm, the scientific goal of every study must be well justified and likely to be achieved through the planned experiment. However, in every situation, the optimal endpoint both minimizes harm to the animal and is consistent with the scientific goal. When the experimental goal cannot be accomplished without causing unacceptable animal harm, the experiment is not adequately justified and should not be performed. These 2 factors—the scientific goal and the amount of animal harm—must be acceptably balanced. The IACUC (or a similar body outside the United States) holds the responsibility of ensuring that the scientific goals are justified and can be accomplished without causing unacceptable animal harm.

Some experimental harm can often be anticipated in light of the animal species and its genetics, the experimental manipulation, and other features including the environment and the analogous human disease. Age can also fall into this category. As in humans,^{23,58,95} physiologic changes may render old mice less responsive to treatments or more vulnerable to adverse reactions than younger mice (for examples, see references 12, 37, 49, 87, and 99). Therefore, studies using elderly mice may require different endpoints than would be applied in studies using younger mice.

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The bottom line is that the potential for animal harm must be aligned with the attainment of the key scientific objectives for each experiment, and the endpoints must consider both the scientific goals and characteristics of the animal subjects. Studies that use *in vivo* imaging of mice with cancer or infectious disease are good illustrations of this need for balance. For example, images of nude mice made at 69 d after orthotopic implantation of luciferase-transfected human pancreatic ductal adenocarcinoma cells show considerable variation in the extent of disease both within and between groups of control and drug-treated mice.¹⁰⁴ However, such images could also represent 2 strains of mice, one more resistant and the other more susceptible, or, relevant to the topic of this article, old mice and young mice. The variation within and between groups raises the question of determining the best endpoint for mice in this study. The scientific endpoint could be a time interval after tumor implantation or drug administration, but clearly using time alone allows some mice to become far more affected than others. Perhaps a marker related to disease severity in individual mice, such as target luminescence or the presence of metastatic disease, would be an adequate endpoint for determining drug efficacy. *In vivo* imaging offers substantial utility for endpoint determination that perhaps has not yet been explored sufficiently.

Similar questions about scientific endpoints arise in infectious disease models. For example, inoculation might initially produce a localized infection at the site of inoculation, followed by localized organ infection or generalized sepsis.^{52,72,102,106} In all cases, the maximal severity of disease that is necessary to achieve the scientific goals should be delineated. For example, a study that focused on sepsis might require the most severe degree of disease, whereas the least severe stage of infection might be sufficient for studies that focused on colonization. Furthermore, differences in the degree of infection or inflammatory damage might be influenced by different strains of mice that vary in their susceptibility to the organism, by differences in the efficacy of various formulations or routes of administration of a drug therapy, by the environment, or by the age or sex of the animals, to list a few example factors.^{5,20} In each case, the minimal severity of infection necessary to achieve the scientific goals must be balanced against the maximal level of infection that will be tolerated for humane reasons.

Studies that use aging mice often have the goal of studying longevity or lifespan. Such studies may focus on healthy aging (the health span) or interventions to extend lifespan.^{28,60} By definition, these types of studies may require that subjects are followed until either spontaneous death occurs or euthanasia is deemed necessary due to the severity of clinical signs. For other types of studies, the appropriate endpoint typically is related to the specific experimental goals. For example, a study might focus on how specific processes or responses change with age (cross-sectional and longitudinal studies), including how age affects disease progression or response to treatment. As in many studies that use young mice, the scientific endpoint usually is related to time, a defined event, or a condition in the animal that either occurs or does not occur after a particular intervention. However, studies with old mice must be viewed with the understanding that older mice are likely to deteriorate sooner over the course of a study than are younger mice. Therefore, an important question is how elderly mice can best be monitored to determine when they are no longer appropriate as research subjects for humane or scientific reasons.

Given the increasing age of human populations worldwide and the prevalence of age-related diseases, research on healthy aging and age-related disease is becoming increasingly

common, and the importance of such work is becoming increasingly recognized. Such research requires the use of aged animals, with associated development of strategies for using these animals effectively and humanely.

General Considerations Regarding Health Assessment of Aging Mice

General health monitoring in mice is a routine animal care procedure. Many processes and forms have been developed to standardize and document this type of evaluation, particularly in the context of specific disease models (for examples, see references 11, 19, 35, 46, 48, 66, and 67). Assessment of an animal's condition generally includes features such as physical appearance and observable or easily measured clinical signs (for example, respiration [rate, pattern, effort], ocular and nasal discharge, unprovoked and responsive behavior, and body condition score).^{62,63,91} However, these assessment procedures usually were developed for application to young adult mice, which are the typical subjects used in research;⁴⁵ these approaches may be therefore less appropriate for studies involving aged mice. For example, old mice normally develop signs like ruffled fur and a more rounded general appearance, and they may seem less alert, less active, and less responsive or more tentative in their movements than do young mice. In younger mice, these signs might be a cause for concern, but they may be normal for old mice. As we all recognize, healthy people, as they age, change in appearance and physical abilities in comparison with both younger persons and with themselves at a younger age. Similarly, measured baseline values for healthy older and younger individuals (humans and mice) may differ in terms of activity, body composition, blood values, pharmacology, and many other parameters (for examples, see references 9, 26, 55, 85, and 96). Another important consideration is that older mice used in lengthy studies may eventually show age-related physical decline that would be unlikely in younger mice kept for the same period of time.²⁶ Health assessments of old mice must take into consideration the naturally different baselines and expected age-related decline that will occur in aging mice during lengthy studies.⁶⁰ For all experimental models and subject populations, health assessment procedures should be tested to determine their reliability in detecting health deterioration and signaling the need for humane intervention while supporting the achievement of the scientific goals.

Knowledge of the survival curve for specific strains and sexes of mice is invaluable in estimating the age range that should be considered to constitute elderly mice. Among C57BL/6J mice, for example, animals that are 3 to 6 mo, 10 to 14 mo, and 18 to 24 mo of age have respectively been defined as mature adults, middle-aged, and old, with a 50% survivorship of 28 mo.²⁶ Thus, in this example, 'old' was defined as occurring before the median lifespan; biologically comparable human ages were estimated as 20 to 30 y, 38 to 47 y, and 56 to 69 y, respectively.²⁶ In another study, female C57BL/6J mice were defined at the start of the study as younger adult (161 d, or approximately 5 mo), older adult (566 d, or approximately 19 mo), and aged (839 d, or approximately 28 mo).¹⁰⁰ In the cited study, no deaths occurred in the young mice, whereas approximately 25% and 75% of deaths, respectively, occurred in the older and aged adults, with 50% survivorship occurring at approximately 900 d (approximately 30 mo) of age.¹⁰⁰ In another report, the 25%, 50%, and 75% survival ages for female and male C57BL/6J mice were 26, 30 and 34 mo and 28, 30, and 32 mo, respectively.¹⁰⁵ Knowledge of the survival curve and of age- and strain-related disease

susceptibility⁶⁰ of the mouse strain being used can provide strong evidence for the likelihood of spontaneous death and therefore can guide the necessary frequency of animal evaluation as the animals age. In addition to strain and sex, age ranges associated with high likelihood of death are influenced by the animal environment and by experimental manipulations such as calorie restriction, resveratrol administration, and growth hormone deficiency.^{6,68,105} Knowledge of strain-related or experimentally-induced disease is also critical, particularly to allow distinctions between normal age-related senescence and deterioration or death that is related to a specific disease or health condition.²⁶

The assessment of aging mice also is complicated by the fact that, as in people, the development of age-related decline and death is not uniform in mice. Mouse lifespan varies across animals despite the fact that, within specific studies, the mice are often genetically identical, and they typically have lived their lives in essentially identical environments. Even with this important homogeneity, survival curves show that some genetically and environmentally identical individuals die at a much younger age than others.¹⁰ Therefore, 2 important questions relevant to endpoints in aging mice are 1) can we identify individual mice within an experimental population as they develop age-related deterioration, and 2) can terminal decline and imminent death be predicted in a population of aging mice.

Frailty Assessment in Mice

An approach to answering the question of “Can we identify individual mice within an experimental population as they develop severe age-related deterioration that predicts imminent death?” comes to us from the human literature on aging. A wide variety of potential markers and methods have been used to assess the risk of age-related disease, disability, and death in people.⁵¹ An overarching approach to this goal is the concept of human frailty.^{76,79} Many different definitions of frailty have emerged in human clinical medicine, with no generally accepted definition or standard.^{13,76,81} However, in application, frailty basically is used to ascertain functional age, which is nested within chronologic age. For purposes of the current discussion (that is, as related to endpoints for aged mice), frailty is defined as a high state of vulnerability to poor health outcomes among mice of the same age.^{75,92} We are all familiar with this concept in practice. You, the readers, are likely to know someone of your same age who is in substantially better or worse overall health than you are, or, in other words, someone who is less or more frail than you are. Older people are likely to know others of the same age or younger who have already died. Frailty in people is a topic of great medical and research interest as the population of elderly humans grows. Furthermore, research has shown that in people, greater frailty is associated with greater risk of illness, greater likelihood of hospitalization, and greater likelihood of death,⁶⁵ and, conversely, those who die, relative to survivors, show a rapid decline of functional ability—that is, an increase in frailty—in the months preceding death.⁶⁴ More than 60 approaches have been developed to assess frailty in people, primarily with regard to risk assessment for adverse health outcomes.^{13,21} Two of the tools most frequently used for assessing human populations—physical frailty (that is, the frail phenotype) and deficit accumulation (that is, the frailty index [FI])—have been adapted for use in mice (Figure 1).

Frailty in humans has been linked to sarcopenia (loss of muscle mass), which results in physical weakness.^{27,29,82} These relationships have led to the development of approaches to evaluate neuromuscular health as a measure of frailty in rats

and mice.^{16,39,40,98} The human system for frailty assessment known as the frail phenotype integrates age-related low energy expenditure, low physical activity, nutritional deficits, and loss of skeletal muscle into a pathophysiologic syndrome that leads to functional decline, disease, and death.⁸⁸ A frail phenotype in humans is determined by evaluating 4 physical attributes—weakness, slowness, low activity, and poor endurance—in combination with unintended weight loss.²⁷ These attributes have been translated into mouse terms as grip strength, walking speed, voluntary physical activity, and endurance (Figure 1).⁵⁰ (A similar system has been developed for rats⁵⁹ but is not discussed here.) To implement this assessment, mice are tested periodically by using an inverted cling test to measure grip strength, a rotarod to measure walking speed, and a running wheel to measure voluntary physical activity.⁵⁰ Endurance is assessed as the sum of the performance durations achieved on the grip test and rotarod.⁵⁰ The neuromuscular health span assessment integrates individual scores obtained from the rotarod and grip strength tests with the maximal isometric force produced by an isolated muscle from the test animal.³¹

The frail phenotype assessment may be useful in aged mice in some specific models (for example, neuromuscular or neurologic conditions), but it has several disadvantages, particularly for evaluating large numbers of older mice. First, mice undergo considerable manipulation in the course of the assessment. Such periodic manipulation and accompanying stress could conceivably accelerate the deterioration of older mice. Second, individual housing is necessary to permit use of the running wheel. Finally, the frail phenotype approach is labor-intensive, requires the equipment for animal assessment, and may be challenging to use for routine assessment of large numbers of mice. In view of these considerations, the frail phenotype approach may be useful in some situations yet may be suboptimal for frequent assessment of large numbers of geriatric mice.

The second mouse-adapted approach to evaluating frailty is the frailty index. This approach conceptualizes frailty as a multidimensional risk state that can be assessed according to the number—rather than the type—of health problems that a patient exhibits.⁸⁸ The FI is based on the concept that as subjects age, they accumulate health deficits over time. For purposes of the FI, these deficits can be anything that is considered to reflect patient health—symptoms, abnormal test results, comorbidities, and/or disabilities.⁷⁹ The criteria for an item to be used as a FI deficit are that it is acquired, age-related, associated with an adverse health outcome, and not present in all or nearly all of the patient population (which would make the item uninformative as a marker of decline).⁷⁹ A FI is created by first generating a list of deficits that incorporates all major systems in the body. Several such lists have been developed, standardized, and validated for human patients.¹³ The deficits observed in a patient are summed and divided by the total number of deficits on the list. For example, when a patient has 6 deficits of a total of 60 items on the list, the FI is 0.1. Larger FI values indicate greater frailty, which in turn indicates a greater likelihood of hospitalization and death.⁷⁹ The FI seems to be a better predictor of death than chronologic age.⁷⁹ The highest FI scores in human patients typically are less than 0.7, given that patients are likely to die beyond this point.⁷⁹ Although human versions of the FI can include as many as 80 items, research has shown that a minimum of 30 items seems to be necessary to provide stable values, prevent ceiling effects, and provide sufficient differentiation of frailty status among and within patients.^{78,79} The specific items that are included seem to be less important than the use of at least 30 items that are selected to incorporate all body

Frail phenotype ⁵⁰	Frailty index ^{70, 100}	Clinical frailty index ¹⁰⁰	Physiologic frailty index ³
Based on Fried Frailty Phenotype ²⁷	Based on health-related variables drawn from systems that are known to change with age in people and mice	Based on Rockwood's Frailty Index ^{76, 80}	Based on parameters selected to reflect the status of different health-related physiologic systems, be objective and quantitative and not visual, and be minimally invasive and suitable for longitudinal studies
Assesses weakness, slowness, physical inactivity and endurance	Measures 31 parameters that reflect activity level, hemodynamic status, body composition, and basal metabolic status; modified 8-item noninvasive version based on activity measures and body weight	Assesses the presence and severity of 31 clinical signs that reflect age-related deterioration in major body systems (integumentary, musculoskeletal, vestibular, cochlear, auditory, ocular, nasal, digestive, urogenital and respiratory) and in discomfort, weight, and temperature	Measures 16 (male) or 18 (female) parameters that reflect physical fitness, the cardiovascular system, and blood composition and that also show significant changes within each sex from 26 to 104 wk of age
Cut-offs were established as 1.5 SD below the age-associated mean; frailty was defined as having at least 3 of the 4 criteria below the cut-off	Using 13-mo-old mice to provide reference ranges, values for individual mice were assigned scores of 0, 0.25, 0.5 and 1 based on standard deviation from reference values; scores were used to calculate a ratio that provided a relative frailty score	Deficits were subjectively rated as none, mild, or severe (0, 0.5, and 1); summed scores were used to develop a ratio that provided a relative frailty score	Using 26-wk old mice to provide reference ranges, values for individual mice were assigned scores of 0, 0.25, 0.5 and 1 based on standard deviation from reference values. Scores were used to calculate a ratio that provided a relative frailty score.

Figure 1. Comparison of mouse frailty assessment scales. Modified and expanded from reference 93 with permission.

systems. The assessment presumes that small changes in many different systems accumulate and interact to produce measurable changes in the patient's general condition.

Over the past 6 y, scientists interested in human frailty have developed several analogous approaches to generating a mouse FI.^{70,75,100} (A similar system has been developed for rats¹⁰³ but is not discussed here.) An early version of the mouse frailty assessment used 31 variables related to activity, hemodynamic function, body composition, and basal metabolic state (Figure 1) and showed that a high FI value was associated with cardiac myocyte hypertrophy and contractile dysfunction.⁷⁰ A subsequent modification of that system used the 8 activity-based items, the data for which were collected from videotaped assessment of open-field activity, in combination with body weight.¹⁰⁰ With the modified approach, frailty increased with mouse age but did not provide much differentiation between older adult and elderly mice.¹⁰⁰ Moreover, both the 31-item and 8-item versions required substantial testing of mice, and the complete 31-item panel required blood collection.

An alternative approach, the mouse clinical FI (CFI), relies on observable clinical findings from a 31-item physical exam, rather than on lab or test results, thereby allowing the assessment of individual mice without the need for special instrumentation or extensive manipulation of the animal (Figure 1).¹⁰⁰ Validation studies have shown that the mouse CFI accurately mirrors the patterns of FI that occur in aging human populations.¹⁰⁰ Furthermore, the CFI correlates well with documented physiologic defects in mice, particularly in the cardiovascular system.^{24,41,61,70} A standardized form has been developed and published for the determination of the mouse CFI for research purposes.¹⁰⁰ Traits on the list are scored as 0, 0.5, or 1 (that is, absent, mild or marked), the scores are summed, and then the total is divided by 31. The resultant score provides a metric that reflects overall

status, without focusing on any specific disease condition. The mouse FI uses 2 quantitative measures—surface temperature and body weight—which are scored relative to deviation from the mean baseline. Skin temperature is measured at the abdominal surface and is one of the most variable measures. Another variable measure is hearing, with variability in part due to the habituation of mice to the noise used; substituting novel sounds at subsequent trials appears to be necessary. Performing the CFI assessment is estimated to require about 4 min per mouse. With training, different raters appear able to use the form with good interrater consistency in most studies^{25,42,100} but not all.⁴⁴

Another assessment tool known as the physiologic FI (PFI) uses 16 objective measures in male mice and 18 measures in female mice; these measures were documented to change significantly as a function of sex and age (26 to 104 wk) in NIH Swiss mice (Figure 1).³ The items in the PFI reflect physical fitness (body weight and grip strength), cardiovascular function (diastolic and mean blood pressure, heart rate, and tail blood flow), and total blood cell composition (WBC and RBC counts and differentials).³ The use of validated items and the objective and quantifiable nature of items confer substantial advantages to the PFI approach. As drawbacks, the PFI requires blood collection and analysis, measurement of grip strength, and restraint and warming to permit hemodynamic measures. These tests potentially could adversely affect natural aging processes and thereby skew values for elderly mice. However, PFI values increased and decreased in association with treatments that were detrimental (obesity) or beneficial (rapamycin administration) to health or longevity.³ Furthermore, PFI evaluation revealed that female NIH Swiss mice show a more rapid accumulation of health deficits than do their male counterparts and that average PFI values are higher in female mice than in male mice of the same age.³ These observations are consistent with many human

	Humans		
	Younger (20–44 y)	Middle-aged (45–64 y)	Older (65+ y)
Age group			
Number	4083	2642	2444
CFI (mean $\pm$ 1 SD)	0.08 $\pm$ 0.07	0.16 $\pm$ 0.11	0.24 $\pm$ 0.13
Range	0.00–0.65	0.00–0.68	0.01–0.77
99th percentile	0.35	0.51	0.59
	Mice		
	Younger (30–299 d)	Middle-aged (300–599 d)	Older (600+ d)
Age group			
Number	251	229	158
CFI (mean $\pm$ 1 SD)	0.05 $\pm$ 0.05	0.21 $\pm$ 0.04	0.29 $\pm$ 0.07
Range	0.00–0.22	0.13–0.32	0.16–0.55
99th percentile	0.18	0.31	0.49

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Human data were extracted from the 2003–2004 and 2005–2006 cohorts of the National Health and Nutrition Examination Survey (NHANES; <http://www.cdc.gov/nchs/nhanes.htm>)

Middle-aged and older mice are those remaining from the initial population of 251 C57BL/6J mice

Figure 2. Comparison of CFI values in adult humans (male and female) and male mice of 3 age groups

studies reporting that at any given age, women on average have greater frailty than men, yet men have higher (younger) mortality than do women.^{18,77,79} Consistent with these observations, potential sex differences should be considered when applying frailty assessment to mice, recognizing that in mice, male lifespan often differs from that of females.^{10,105}

Among these 4 approaches to mouse frailty assessment (Figure 1), the one that has received the most study to date is the CFI. As in humans, the mouse CFI increases with age, indicating that older mice exhibit more clinical signs than do younger mice.¹⁰⁰ In addition, factors that alter lifespan are appropriately reflected in the mouse CFI.⁴³ For example, short-lived mouse strains develop an elevated CFI at younger ages than do long-lived strains. In addition, 2 treatments that are known to increase lifespan in mice—caloric restriction and resveratrol administration—were both associated with lower CFI in experimental mice as compared with untreated mice of the same age.⁴³ Finally, mice that die over the course of the observation period have higher CFI scores than do mice of the same age that remain alive.¹⁰⁰

In Figure 2, CFI data collected from mice are compared with similar data derived from human databases.⁷⁵ These data show that mice are similar to people in several ways. First, the average CFI increases with age in both mice and people, consistent with the accumulation of more deficits in individuals as they age. Second, deficits accumulate variably across individuals, as evidenced by standard deviation and range values; this variation reflects the individual differences that occur in the general overall health status of mice and humans as they age. Finally, the CFI distribution broadens with age and is greatest in the oldest groups. Thus, some individuals remain remarkably robust as they age, whereas others become excessively debilitated (Figure 2).

The data in Figure 2 also reveal differences between people and mice. First—and maybe most importantly—CFI values in mice are relatively less variable than those in people. This feature is apparent in terms of both standard deviations and ranges; it suggests that mice are more consistent than people in their age-related decline. This finding is probably not surprising because, as compared with people, inbred research mice are a highly homogeneous population, with identical genetics and environments, a known history, and controlled interventions. Therefore, mice are likely to develop age-related changes in a more uniform and predictable manner than are people. Second, the 99th percentile CFI value is lower in mice than in people in all age groups, indicating that mice die with a relatively lower

CFI than do people; in other words, as deficits accumulate, they are relatively more lethal in mice than in people. This difference may be related to therapeutic interventions that are administered to aging people but not to aging mice.

Finally, the authors of the cited paper⁷⁵ comment in the discussion that when clinical veterinarians said that a mouse should be euthanized, the CFI scores for those animals were between 0.50 and 0.54. A 99th percentile value of 0.49 for mice indicates that 99% of the mice had values that were 0.49 or lower. Because the CFI range extends to 0.55, it appears that 1% of these mice—those with FI values above 0.49—could be candidates for euthanasia. This relationship appears to both validate the veterinary clinical assessment for accurate detection of severe debilitation and shows that the clinical assessment can be quantified and standardized to provide a value that warrants euthanasia or indicates that the mouse should be monitored more frequently. This evidence-based documentation provides objectivity to veterinary clinical impressions and therefore has the potential to support veterinary efforts to intervene in the disposition of individual mice. However, an important point to remember is that the CFI is designed to reveal chronic and gradual deterioration that occurs with age. Therefore, reliance on CFI for endpoint determination may reduce, but not prevent, spontaneous and unexpected death in aging mice.

A recent study comparing the mouse frail phenotype and CFI approaches concluded that—as is the case for comparisons of the comparable human systems^{36,54,101}—the 2 mouse systems do not identify the same mice as frail.^{42,93} However, the CFI identified more mice within the population as frail, and CFI scores were correlated with changes previously associated with aging in mice.⁴² Standardized or comprehensive frailty assessment of mice with genetic predisposition to accelerated aging has been conducted to a limited extent.^{93,94} An additional, recently proposed approach for the evaluation of geriatric mice purports to measure resilience rather than frailty.⁸⁴ This approach exposes mice to 3 challenges—cold water immersion, sleep deprivation, and cyclophosphamide administration—and measures the time to recovery of normothermia, performance on a radial water maze combined with blood glucose concentrations, and rebound neutrophilia, respectively, as measures of physiologic resilience.⁸⁴ However, as with the frail phenotype, this approach to resilience assessment has the disadvantages of being labor- and equipment-intensive and of potentially altering outcomes by exposing mice to significant stressors.

An important caveat relevant to mouse frailty assessments is that they were developed as a model for the study of human frailty and for associated research on aging, lifespan, and health span^{50,69,75,100} rather than for endpoint determination. Clearly, some attributes of the mouse CFI suggest that it could be used to determine endpoints, particularly because the values correlate with the veterinary clinical assessment regarding the need for euthanasia.⁴² However, frailty assessments could be refined for endpoint determination by establishing validity for signaling the need for intervention in studies of geriatric mice. Principal component analysis of CFI data might highlight those items that contribute most to increasing the FI of aging mice and to its relationship to imminent death, thereby potentially streamlining the list of items. In addition, different experimental models may produce health changes that are specific to the model. Such symptoms would likely be useful additions to the FI assessment of mice used in such studies. Clearly, a wide range of developmental and validation studies would be valuable in translating the mouse FI into a tool for endpoint assessment.

In summary, like old people, old mice accumulate deficits that lead to impairment and eventually death. Therefore, the experimental design must include a plan for recognizing and managing age-related deterioration in geriatric mice used in research studies. In particular, a clinical sign or deficit that would be unusual and trigger concern in a young mouse might be normal yet not a critical health concern in an old mouse. Understanding the concept of age-related frailty is fundamental to the ability to conduct meaningful research on elderly mice.

Can Terminal Decline and Imminent Death Be Predicted in Aging Mice?

A complementary approach to determining overall animal health status based on many diverse variables, as in the FI, is to identify a few specific markers that might offer more precision and easier implementation. This strategy has been used to identify practical and objective criteria that can predict imminent death in mice. 'Practical' and 'objective' are important descriptors for this strategy. Subjective criteria are often incur bias, particularly when the raters are not blinded to experimental groups. The ambiguity associated with subjective criteria can contribute to reluctance to euthanize an animal or otherwise intervene. The use of validated objective criteria may minimize this tendency. Practicality is crucial to allow relatively large numbers of mice to be evaluated in a timely manner. In light of these criteria, published research has focused on monitoring body weight and temperature in mice. Similar data are available for rats but will not be reviewed here.^{47,56,57,83}

Several investigators have studied temperature regulation in aging mice, although not initially for purposes of endpoint determination. An early study of this type was designed to evaluate age-related maintenance of temperature homeostasis.⁷⁴ In that study, the research team performed weekly measurements of rectal temperature in C57BL/6J mice beginning at 25 mo of age and found a significant decrease in rectal temperatures during the 5 wk prior to death.⁷⁴ The use of rectal probes to measure temperature was perhaps the best technique available for mice in the 1980s. However, rectal probes may be less accurate than more modern means of measuring temperatures, are certainly stressful to use in mice, and may cause injury.

A subsequent study of homeostasis in aged mice used telemetry to measure locomotor activity, core temperature, and heart rate in male AKR/J mice.⁸⁶ In the cited study, 25 mice were implanted with abdominal transmitters at about 190 d of age; the

median lifespan of these mice was 314 d (range, 219 to 470 d). Data were collected only on weekends to conserve transmitter battery life. This schedule allowed the generation of 2 d of circadian patterns every week without disturbing the mice. A comparison of values collected from mice a few weeks after implant surgery, when they were still relatively young and healthy, with values from the same mice during the 2 to 3 d before they died revealed 2 important changes. First, the mice showed much higher values for all 3 measures—activity, temperature, and heart rate—when they were younger as compared with values measured a few days before death. Second, the mice showed a marked absence of circadian variation as they approached death. Other researchers also have reported age-related changes in circadian rhythms, including reductions in the circadian amplitudes of temperature and running wheel activity (reviewed in reference 53). Furthermore, studies of the circadian rhythm of body temperature in aged rats have found that although some of them maintain normal circadian rhythms of body temperature, others develop lower temperatures, flattened rhythms, or even complete arrhythmia,⁴⁷ with severe circadian disorganization occurring a few days before death.²

This study⁸⁶ examined these temporal patterns in relationship to death by synchronizing data according to days preceding the day of death. Data from individual mice showed that both heart rate and body temperature were relatively stable for most of the animal's life but fell precipitously during the 3 wk that preceded death. Therefore, data from individual mice, when plotted evaluated as a function of days preceding death, indicate that health deterioration begins weeks before death occurs, with decreases in values and loss of rhythms indicating the development of severe physiologic dysregulation. The approach used in the described study⁸⁶—telemetry—provides a wealth of information, including circadian rhythms, but is problematic for routine animal monitoring because it requires surgery, costly equipment, and either individual housing or complicated schedules to monitor individual mice.

More recent work has used a third and relatively noninvasive approach to monitoring mouse temperature.⁸⁹ In this approach, a chip transponder that allows remote measurement of temperature was implanted subcutaneously between the scapulae, allowing temperature and identification number to be read by simply passing a wand over the mouse. Every week at the time of cage change, each mouse was identified, and its temperature was measured (often requiring tail restraint) while the mouse either remained in the home cage with its cage mates or was placed on the cage grid top. The mouse was then weighed and placed in its clean cage. Thus, mouse manipulation and the time required to collect the data were minimal. The studies were conducted on male and female AKR/J mice and male C57BL/6J and BALB/cByJ mice; Figure 3 shows the survival curves for these mice.⁸⁹ AKR/J mice generally are considered to be the shortest-lived strain, whereas the other 2 strains live as much as 3 times longer.¹⁰⁵ The figure illustrates the variation in lifespan between strains as well as variation within genotype and sex. The key question is whether these deaths could be predicted.

The first column of panels in Figure 4 shows values that were measured for body weight and temperature during the 13 wk prior to death in male and female AKR/J mice. The product of temperature and body weight (TxBW; Figure 4, lower panels) was used to incorporate the information from both measures into a single value. As in the telemetry study of male AKR/J mice,⁸⁶ both male and female AKR/J mice showed a precipitous fall in temperature and body weight during the 3 wk preceding death.⁸⁹ For use in practice, values for each mouse or for

Figure 3. Kaplan–Meier survival plots of AKR/J, C57BL/6J, and BALB/cByJ mice. The horizontal dashed line indicates the point at which 50% of the mice remained alive. Reproduced from reference 89 with permission.

groups of mice can be standardized (Figure 4, panels labeled ‘Normalized Values’) based on the average of several consecutive weekly values that were collected when body weights were stable (Figure 4, circled values on left panels). If weight change is anticipated (for example, growth or obesity), then a rolling average can be used (for example, the average of weeks 10 through 13 prior to the current calendar date). Developing a standard for each mouse allows control for variation in weights of individual mice. When using subcutaneous chips, temperature values are generally less variable among mice than are body weights. Some studies of temperature as a marker for euthanasia have used infrared thermometers to measure surface temperatures.^{1,8,33,97} Comparison of surface thermometers with chip transponders suggests that the surface temperatures may be less reliable.³³

Individual standardization allows thresholds to be established for each mouse based on its own values. In the data shown in Figure 4, thresholds of a 5% decrease in body weight and a 1 °C fall in body temperatures or a 5% decrease in T×BW value seemed reasonable for predicting imminent death. Values for specific strains or models can be established in preliminary studies such that, in subsequent studies, values below these thresholds predict with a high degree of confidence that death would occur in a few weeks or less. The rightmost panels in Figure 4 show data from male C57BL/6J and BALB/cByJ mice.⁸⁹ As in AKR/J mice, these 2 strains also develop a fall in temperature, body weight, and T×BW values in the weeks preceding death.⁸⁹ Another study found a similar pattern of decline in temperature, as well as in locomotor activity, in an inhouse colony of BALB/c mice.⁷ However, the 3 strains of mice (AKR/J, C57BL/6J, and BALB/cByJ) show pronounced differences in their patterns of decline, with AKR/J mice showing a precipitous fall in values and the other 2 strains declining more gradually.⁸⁹ The rate of decline must be considered in determining how to standardize values for each mouse; for example, rolling averages from 13 to 16 wk prior to a specific study date could be used for normalization in C57BL/6J and BALB/cByJ mice, whereas an earlier period from 10 to 13 wk could be used for AKR/J mice. Alternatively, all strains could be normalized against stable adult values for each mouse. The sharp declines in the AKR/J mice as compared with the other 2 strains may be related to the high incidence of leukemia and lymphoma as the

cause of death in AKR/J mice,^{17,30,71} as compared with more varied and perhaps less acute causes of death in the other 2 strains. Another consideration is that mouse strains like AKR/J, which have specific diseases or genetics that lead to reduced lifespans, are likely to succumb due to aging mechanisms that are related to their specific disease rather than to mechanisms that underlie normal aging and senescence.^{32,34}

Although temperature and body weight appear to be useful markers for predicting death in mice, an important related question is how changes in these values correspond to the development of a moribund state and clinical indications for euthanasia. This question was addressed by studying outbred Hsd:ICR mice (retired breeders, approximately 8 mo old at the time of procurement); temperature and body weight were measured at 1- to 4-wk intervals (more frequently as the mice aged).⁷³ Mice were declared moribund and euthanized immediately when they displayed any of the following signs: palpable hypothermia, lack of response to manipulation, unwillingness or inability to walk, ulcerated tumors, or severe dyspnea or cyanosis. The data show that mice that became moribund and were euthanized followed the same general time course of T×BW decline as did those mice that died spontaneously (Figure 5). An important point inherent in this finding is that mice that died spontaneously did not first become moribund—they were simply found dead in the cage at morning health checks, without any obvious preceding clinical signs. However, the temperature and body weight values did provide physiologic signs. The mice appeared to show a 3-stage pattern in both temperature and body weight measurements, comprising a relatively stable period, an initial gradual worsening, and a final more rapid deterioration that preceded death by a few weeks. On a group basis, the values from the mice that became moribund and were euthanized correlated closely with the values from the mice that died without becoming moribund.⁷³ Using the markers of temperature and body weight, therefore, gives predictive objectivity to the assessment of individual animals, making it easier to determine and justify if and when euthanasia of individual mice should be performed, even among those mice that do not become overtly moribund. Using a threshold of 90% of stable T×BW values would permit prediction of death in the described ICR mice⁷³ to within 2 wk, thus representing about 2% of the total lifespan.

The use of validated predictive markers for imminent death has 2 major advantages. First, with regard to benefitting the science, validated preemptive euthanasia allows terminal data collection, including collection of blood and tissue samples. In addition, development of a moribund state can influence data collected from those animals, for example by creating hypoxic conditions in the tissues. Preemptive euthanasia would avoid such confounding factors. Second, preemptive euthanasia avoids any terminal distress the animal might suffer in the process of dying.

In general, collecting data on both temperature and body weight seems to be sufficient for predicting death in aged mice, at least under some circumstances, and these data are simple to collect. In addition, this approach has shown utility for predicting death in young mice, notably in relatively acute studies of infectious disease, toxicity, and irradiation (for examples, see references 1, 15, 22, 33, 46, and 90). In these acute studies, the 2 markers can predict death within a period of days or even hours. However, in aging studies, these markers provide a prediction of 2 or 3 wk in advance of death. This time frame may be useful when considered in the context of a 3- or 4-y normal lifespan for a given mouse strain. However, any system or markers intended to assess animal health and serve as an endpoint must

Figure 4. Body weight, temperature, and temperature $\times$ body weight during the final weeks of life in AKR/J, C57BL/6J, and BALB/cByJ mice. For all panels, values of temperature, body weight, and the product of temperature and body weight (T $\times$ BW) were aligned with regard to the day of collection prior to death. The left panels show the mean of actual measured values for male (filled circles) and female (open circles) AKR/J mice. The horizontal lines represent the average of all values collected during weeks 9 through 12 prior to death for male (solid line) and female (dashed line) mice and were considered to be the baseline. Stars denote values significantly (P value < 0.05) different from the baseline value for female (open stars) and male (filled stars) mice. In the center panels, values for individual AKR/J mice were normalized with regard to each animal's individual baseline values, defined as the mean of values collected during weeks 9 through 12 prior to death for that mouse (circled values). For body weight and T $\times$ BW, data at each time point were converted to a ratio of the baseline value for that mouse; the horizontal solid and dashed lines on those panels respectively indicate ratios of 1.0 and, as a visual benchmark, 0.95. For temperature, data were expressed as a difference from the baseline value for that mouse; the horizontal solid and dashed lines on that panel respectively indicate normothermia and, as a visual benchmark, hypothermia of 1 °C. In the right panels, data from C57BL/6J and BALB/cByJ mice are displayed in a similar manner to those of AKR/J mice in the center panels. However, for the C57BL/6J and BALB/cByJ mice, values for individual animals were normalized with regard to each animal's individual mean baseline values during weeks 13 through 16 prior to death (circled values). For body weight and T $\times$ BW, data at each time point were converted to a ratio of the baseline value for that mouse; the horizontal solid and dashed lines on those panels respectively indicate ratios of 1.0 and, as a visual benchmark, 0.95. For temperature, data were expressed as a difference from the baseline value for that mouse; the horizontal solid and dashed lines on that panel respectively indicate normothermia and, as a visual benchmark, hypothermia of 1 °C. Open and filled stars denote values significantly ($P < 0.05$) from the baseline value for BALB/cByJ and C57BL/6J mice, respectively. Modified from reference 89 with permission.

be validated for each model and applied in a manner that is consistent with the scientific goals of each study. The definition of 'imminent death' will differ across studies, as will the time frame in which that prediction is useful. For example, in studies designed to measure maximal lifespan, a change in lifespan of as little as a few days may be significant when averaged across

an entire study population. In such cases, euthanasia of animals within a few of weeks of natural death potentially could obscure small—but real—effects of the variable of interest. However, as opposed to euthanasia, declining temperature and body weight could be used to trigger closer and more frequent evaluation of individual animals in anticipation of the development of a

Figure 5. Body weight and temperature in the last weeks of life of Hsd:ICR mice. The panel represents the average of the calculated values of temperature $\times$ body weight (T $\times$ BW) for individual mice. The circle highlights values that were averaged to generate the 100% value for each mouse, represented by the upper horizontal dashed line. In the last 2 wk of life, values fell to below 90% of the 100% benchmark (indicated as the lower dashed line). Reproduced from reference 73 with permission.

moribund state (for example, palpable hypothermia, unresponsiveness to handling, unwillingness to move) which, in turn, would trigger immediate euthanasia and necropsy.

For some types of studies, markers other than or in addition to temperature and body weight might be useful. For example, studies of neuromuscular or neurodegenerative disease could include periodic measures of grip strength or open field activity. However, a general recommendation is that any additional markers that might be considered should be objective. In addition, as with frailty approaches, the T $\times$ BW approach will not capture imminent death in all subjects; even with careful monitoring, some mice will develop illness that causes acute death in a time frame that precludes detection by weekly or even daily monitoring. Therefore, models or mouse strains at risk for sudden death may benefit from more frequent assessment.

Summary

Perhaps the most fundamental concepts presented in this review are the need to recognize that age is associated with declining health and that studies of aging mice may require a different norm for animal evaluation than might be appropriate for studies involving young mice. Methods to assess the health status of aging mice must recognize that animals will change in appearance, behavior, and physiology in association with normal but healthy aging, and signs that might be troubling in a younger animal may be totally normal in a geriatric animal. Increasing frailty should be expected in elderly mice, just as it is in elderly people. This perspective must be considered when developing endpoints for studies using geriatric mice. Therefore, objective criteria can facilitate the assessment of health during aging in mice.

Another fundamental concept is that any systems or markers that are selected as endpoints for any study or model must be validated, interpreted, and implemented in the context of the scientific goals of the study. If the implementation of an endpoint marker prevents the collection of essential data, then either an alternative endpoint must be identified, or the justification for the study must convince the IACUC or comparable oversight body that the expected animal harm is acceptable due

to the importance of the resultant findings. Any study that is conducted must accomplish the goals of the study while causing only adequately justified animal harm.

Although numerous scoring methods have been developed to assess rodent health status, most of these were designed for specific types of research studies using young adult mice. These systems may not be ideally designed or validated for health assessment of aging animals. However, validation studies have shown that mouse CFI scores above a specific threshold are highly correlated with veterinary recommendations for intervention in old mice. The FI approach may be useful for evaluating overall health during aging in the context of identifying humane endpoints. However, a disadvantage of the FI approach is that it may not be realistic or even feasible for studies involving large numbers of animals. A typical CFI assessment takes about 4 min per mouse, which may limit its use to studies of relatively small numbers of mice. However, principal component analysis of FI data potentially could highlight those items that contribute most to increasing the FI of aging mice in relationship to imminent death and in that way perhaps streamline the list of items that are relevant for predictive purposes.

The approaches and parameters discussed in this review vary substantially in terms of effort required, need for specialized equipment, and invasiveness. The feasibility and practicality of using any of these measures should be evaluated with regard to the goals of the study, the available equipment, and the number of animals being monitored. Different assessment systems may be warranted for small-scale hypothesis-driven studies as compared with large-scale colony management. The important issues are to have an effective system in place and to rigorously adhere to that system.

Studies focused on temperature and body weight have found that these 2 parameters can indicate physiologic decline in advance of death in a useful time frame for studies of both aged mice, as reviewed here, and acute mouse models of infectious disease and toxicity. Both of these parameters are measured as part of the mouse CFI, but their individual and joint contributions to worsening FI scores have not been reported. Initial decreases in temperature and body weight begin several weeks prior to death, thus giving research and veterinary staff early indications that close monitoring is warranted and that eventual intervention will likely be needed. These 2 markers appear sufficient to predict imminent death in mice in several strains and experimental models, but other markers (for example, grip strength) may be informative for particular models. The ability to predict imminent death is particularly useful for mice that do not appear moribund because the data signal when closer monitoring or preemptive euthanasia should be performed, thereby avoiding terminal suffering in the mice by triggering a timely and humane end to the experiment. In addition, timely preemptive euthanasia allows antemortem collection of data and samples that would be lost if the mouse died unexpectedly and perhaps would even be compromised if the animal was moribund at the time of euthanasia.

An additional consideration relevant to endpoints in geriatric mice is the possibility of correctable factors that could lead to death if uncorrected. Examples include malocclusion, dermatitis, and large or ulcerated superficial tumors. Some environmental factors, such as water leaks and aggression, also fall into this category. A strategy for managing such conditions, consistent with the scientific goals and the minimization of animal harm, should be incorporated into the research plan during the protocol development stage.

Perspectives

Successful implementation of all endpoint strategies requires 3 features: personnel training, a well-defined system for animal assessment, and clear lines of responsibility and authority. Personnel must be well trained to assure that everyone understands what the endpoint is and knows how to identify it. Measures should be taken to test and assure consistency among personnel in evaluating the endpoint criteria. The more subjective an assessment is, the more important is its consistent evaluation. In addition, whenever possible personnel should be blinded with regard to animal treatment. Failure to blind personnel doing the assessments can result in bias and unequal application of criteria across groups.

Second, to the extent possible, the endpoint should be unambiguous in terms of triggers and actions. Furthermore, the triggers and actions should be written and implemented as written. For example, assessment forms should include clear action items—if A occurs, then do B. When a given score or status is reached, actions could include closer or more frequent observation, medical intervention, removal of the animal from study, or euthanasia.

Finally, everyone involved in the study should be absolutely clear about who, in addition to the veterinarian, can make the decision to intervene. The more objective and validated the criteria are, the less of a problem deciding whether and when to intervene is likely to be. Particularly in longevity studies, and perhaps in other types of studies that used geriatric animals, the research team can be reluctant to interfere with or euthanize an animal. The need for students and staff to contact the project director or another individual before taking action creates confusion and delay and should be clarified ahead of time, perhaps in the protocol approval process.

Another ancillary point is that the data that are collected for endpoint evaluation should be viewed as part of the experiment. Data relevant to endpoint markers that are used in the context of specific models should be collected, analyzed, validated, and published. Viewing the collection of endpoint data as a component of the experiment can help the research team to identify the most informative markers, thus perhaps refining and simplifying the process of endpoint evaluation for future studies. Dissemination of this type of information to the scientific community gives the entire field the opportunity to consider and benefit from what has been learned.

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