January 31, 2006

Alan M. Goldberg, PhD
The Johns Hopkins Center for Alternatives to Animal Testing
111 Market Place, Suite 840
Baltimore, MD 21202-6709

Dear Dr. Goldberg,

I am pleased to forward the final report of our study entitled “Use of Body Condition Scoring as an Endpoint for Tumor Growth Studies.” As you will be able to see from your review, we were able to develop some very useful conclusions regarding the use of body condition scoring to enhance the monitoring of animal well-being. We are excited about the opportunity to share these results and look forward to presenting and publishing our findings in appropriate venues.

Sincerely yours,

Debra L. Hickman, DVM, MS, DACLAM
Use of Body Condition Scoring as an Endpoint for Tumor Growth Studies

Debra L. Hickman, DVM, MS, DACLAM
VA Medical Center, Portland, OR

Abstract

When mice are used in studies where wasting and death are potential complications, practical, rapid, and noninvasive methods for assessing health status and establishing endpoints are needed. Tumor growth studies are often very challenging as the growth of the tumor may mask one of the more commonly used endpoints – loss of weight. In 1999, body condition scoring (BCS) was characterized as a means of evaluating the health of normal mice as BCS correlated well to white blood cell count. This project proposed to evaluate BCS as an endpoint for tumor growth studies, used in conjunction with other common endpoints such as weight loss, general body condition, attitude, tumor size, and the presence of ulceration on the tumor. In keeping with the principle of reduction of overall animal use, this project characterized the use of BCS in collaboration with ongoing cancer research at our institution. This study showed that body condition scores can be a beneficial addition to the health monitoring for animals that are used in studies with tumors that are intra-abdominal, such as ascites and other bulk producing tumors of the abdomen. However, this study also demonstrated that body condition score is not appropriate for all studies of tumor growth. The skin tumor growth studies that we monitored had no significant changes in body condition scores that were predictive of morbidity or mortality, and more traditional clinical signs were found to be reliable for this model. This study confirms that body condition scores are a valuable adjunct to clinical health monitoring. Additional applications should be considered in the future.
Introduction

When mice are used in studies where wasting and death are potential complications, practical, rapid, and noninvasive methods for assessing health status and establishing endpoints are needed. Endpoints typically consist of a panel of clinical observations that suggest that an animal is in pain or distressed, requiring intervention through treatment or euthanasia. Tumor growth studies are often very challenging as the growth of the tumor may mask one of the more commonly used endpoints—loss of weight.

In 1999, body condition scoring (BCS) was characterized as a means of evaluating the health of normal mice (Ullman-Cullere, 1999) as BCS correlated well to white blood cell count. The researchers concluded that BCS has potential as an endpoint for experimentally manipulated mice, such as those on tumor growth and ascites studies, but that formal evaluation of this application should be pursued. To date, follow-up studies have not been published. This project proposed to evaluate BCS as an endpoint for tumor growth studies, used in conjunction with other common endpoints such as weight loss, general body condition, attitude, tumor size, and the presence of ulceration on the tumor.

In keeping with the principle of reduction of overall animal use, this project characterized the use of BCS in collaboration with ongoing cancer research at our institution. We were able to evaluate the use of BCS as an adjunct measure of animal health and well-being for two different tumor growth studies: subcutaneous skin tumors and intra-abdominal or intra-thoracic tumors. The results of these studies are presented separately below.

Materials and Methods

Environment. The mice were housed in polycarbonate shoebox cages with filter tops (Thoren Caging Systems, Hazelton, PA) and corn cob bedding (Bed-O'Cobs, Maumee, OH). Cages were changed at least once weekly in a laminar flow changing station (Lab Products, Seaford, DE). The animal caretakers wore gloves while changing cages and sprayed their gloves with a 10% bleach solution between each cage. Soiled cages were sanitized in a mechanical cage washer with a final rinse temperature of 180°F (82°C). The room was kept on a 12:12-h light:dark cycle, and animals were provided rodent chow (LabDiet, St. Louis, MO) and tap water ad libitum. Temperature and humidity were maintained at 72°F (22°C) and at least 30%, respectively. The Portland VA Medical Center Institutional Animal Care and Use Committee approved all projects using these mice in accordance with applicable federal regulations.

Indirect exposure sentinel mice were used to screen the colony for pathogens on a quarterly basis. Sentinel mice were five-week old female colony or ICR (Taconic, Germantown, NY) mice that had been exposed to pooled dirty bedding from colony cages for a minimum of 21 days. Serum samples collected from these sentinel mice by cardiac exsanguination under isoflurane anesthesia were submitted to the University of Missouri Research Animal Diagnostic Laboratory (RADIL) for serologic testing. Internal and external parasite screens were performed in-house. At the time of this clinical investigation, these lines of mice were determined to be free of mouse corona virus, Sendai virus, mouse parvovirus, minute virus of mice, ectromelia virus, reovirus type 3, pneumonia virus of mice, murine adenovirus, Mycoplasma pulmonis, lymphocytic choriomeningitis virus, mouse rotavirus, mouse encephalomyelitis virus, polyoma virus, murine cytomegalovirus, and rodent pinworms and mites.
Monitoring. For all studies, each mouse was assigned a unique number. At least two observers, blinded to the treatment of the mouse, were assigned to score the mice at the intervals described below. All mice were scored on the same day, with each observer completing their scoring independently and within 6 hours of the other. The scoring involved weighing of the mouse, measurement of tumors (if visible), palpation to assign body condition score, and characterization of the mouse’s attitude (“curious” or “lethargic”) and posture (“normal” or “hunched”). These scores were entered into a spreadsheet for further analysis. A new score sheet was used each day to blind the observers to the values they had assigned to a particular mouse previously.

To assess body condition score, the mouse was placed on a flat surface (e.g. wire bar lid). Holding the base of the tail with the thumb and index finger of one hand, the degree of muscle and fat cover was assessed and scored by palpating the sacroiliac region and referring to the chart provided (Figure 1). In our experiment, we used a “+” to indicate gradients between scores.

Figure 1: Body condition score chart (Ullman-Cullere, 1999).
Data Analysis. Initially, the scores assigned by the two observers for each individual mouse were compared by analyzing two-tailed z-tests of the differences between the two observers. Comparison of weights and body condition scores were performed for each animal by calculating the difference between initial and final monitored parameters. Analysis of variance was performed between treatment groups (mice treated to develop tumors versus mice not treated to develop tumors). Significance was determined through comparison of means and confidence intervals between groups.

Subcutaneous Skin Tumors

Mice. Two studies of mice were used. In the first study, mice received topical application of dimethylbenzanthracene (DMBA) and 12-0-tetradecanoylphorbol-13-acetate (TPA) to induce skin tumor growth. In the second study, transgenic mice that spontaneously developed skin tumors were monitored. For both of these studies, the investigator was attempting to characterize rates of metastatic conversion and the effect of dietary manipulation. All mice were at least 8 weeks of age and had developed at least one tumor when enrolled in this study. Mice were maintained until single tumors were 10% of body weight or until clinical signs of morbidity (e.g. anorexia, hunched posture, non-responsive to outside stimuli) were identified. At study termination, they were euthanized by carbon dioxide asphyxiation for tissue collection.

Monitoring. As the tumors did not change significantly over a 5-7 day period, the monitoring interval for these studies was set at weekly. Mice were monitored as described above. The total number of tumors was also collected, as mice in these studies developed multiple papillomas (Figure 2). The size of the largest tumor was recorded for additional analysis.

![Figure 2: Representative example of mouse with skin papillomas, utilized for the subcutaneous tumor phase of this study.](image)

Analysis. Statistical analysis was performed as described above.
Intra-Abdominal and Intra-Thoracic Tumors

*Mice.* For the intra-abdominal tumor study, 7 week old female C57BL/J mice were inoculated intraperitoneally with \(5 \times 10^3\) C6VL tumor cells. They were next treated with monoclonal antibodies to the C6VL tumors to assess survivability, as per the IACUC approved protocol of our collaborator. For the purposes of this study, mice were maintained until they showed clinical signs of morbidity (e.g. anorexia, hunched posture, non-responsive to stimuli), then they were humanely euthanized by carbon dioxide asphyxiation for tissue collection.

For the intra-thoracic tumor study, 7 week old female C57BL/6J mice were infused with \(7 \times 10^5\) melanoma cells via intravenous injection to induce lung metastases. They were next treated with a *Listeria* based vaccine designed to prevent metastasis of the melanoma cells, as per the IACUC approved protocol of our collaborator. For the purposes of this study, mice were to be maintained for 19 days after melanoma cell infusion, then euthanized by carbon dioxide asphyxiation for tissue collection.

*Monitoring.* All of the intra-abdominal and intra-thoracic model mice were scored three times per week. As tumors were not visible, no additional parameters regarding tumor size or character were recorded.

**Results**

The data collected for the mice with induced pulmonary tumors was discarded. The mice were euthanized by the primary investigator before they developed any evidence of clinical disease that this study could evaluate. The data collected from the skin and abdominal tumors are presented below.

Initially, samples of all recorded parameters by each observer were compared to determine reproducibility of the body condition score technique. The differences between each observer’s weight and body condition score record were analyzed through examination of the man and confidence intervals and z-tests (Microsoft Excel and JMP). For the mice on the abdominal tumor study, weight had statistically significant between observer variation (\(p<0.05\)). For the mice on the skin tumor study, weight had no significant between observer variation (\(p=0.1013\)). Body condition scores for both studies were found to have statistically significant between observer variation (\(p<0.05\)). However, the difference was found to be \(0.08 \pm 0.024\) (mean ± CI). Since the scoring interval was 0.5, this difference was found to be inconsequential for practical use of the technique.

**Subcutaneous Skin Tumors**

Comparison of the initial and the final weight and body condition scores showed no significant difference over a 9-week monitoring period (Figure 3). Additionally, within group analysis of variance was performed for body condition score, weight, tumor size, and number of tumors. No correlations of statistical significance were noted.
Intra-Abdominal Tumors
Comparison of the initial and final weights and body condition scores showed that the weights increased while the body condition scores decreased (Figure 4). One-way analysis of variance was used to analyze the difference between control and treatment groups. A within group comparison was also made for weights and body condition scores of animals euthanized versus animals that died. This analysis showed that the means and confidence intervals for both groups were nearly identical (Figures 5 and 6) suggesting that body condition score could reliably be used to intervene before morbidity progressed to mortality. Mice that developed intra-abdominal tumors showed an increase in weight of approximately 16% from initial when the initial and final weights of treated animals were compared to the control group (“continue” in Figure 5). These mice also displayed a significant decline of at least one body condition score when the initial and final body condition scores of treated animals were compared to the control group (“continue” in Figure 6).
Figure 5: One-way analysis of the weights recorded by each observer (a and b). Note that the means and confidence intervals are nearly identical for the mice that died or were euthanized. There is significant difference between the dead or euthanized and the control (continue) groups.
Figure 6: One-way analysis of the weights recorded by each observer (a and b). Note that the means and confidence intervals are nearly identical for the mice that died or were euthanized. There is significant difference between the dead or euthanized and the control (continue) groups.
Discussion

The analysis of differences between observer assigned body condition scores and weight collection was of interest for demonstrating consistency of the techniques. The analysis was made with the assumption that there would be no difference between observers. The analysis revealed that there were differences between observers in weights and body condition score parameters, but no significant difference between measurements of tumors on animals in the skin tumor study. For the weights, the differences were suspected to be due to the interval of up to 6 hours between measurements, suggesting that weights must be consistently collected at the same time each day for appropriate interpretation of observations. The scale that was used for the abdominal tumor project was able to weigh out to thousandths of grams and was very sensitive to “noise” produced by animal movement. For the skin tumor project, a newer, less sensitive gram scale was used, resulting in the non-significant difference between each observer’s record of weights (p=0.9494), suggesting that equipment must also be considered when interpreting apparent weight loss or gain. Weight was not found to be a reliable predictor of animal health due to the number of potential variables that could affect accurate and consistent measurements.

For the body condition scores, the average difference between each observer was 0.08 ± 0.024 (mean ± CI). Although the difference was statistically significant using a two-tailed z-test for analysis, the average difference is less than the 0.5 intervals of the body condition scoring system, suggesting that the difference is not of practical concern. This data supports the conclusion submitted by Ullman-Cullere (1999) that the technique is reproducible and consistent between individuals.

When the data from each observer was analyzed independently, no significant correlations were noted for body condition score, weights, and tumor size for the animals used on the skin cancer studies. We found that the use of body condition scoring as an adjunct means of monitoring well-being of animals where skin tumors have been induced was unrewarding. Despite the presence of up to 20 papilloma tumors on individual mice, the body condition score did not decrease in advance of other parameters, such as tumor size or ulceration. Although we saw one mouse exhibit a decline in body condition score once a tumor became ulcerated, a consistent decline in body condition score in all mice was not recognized. Clinically, ulcerated tumors became larger and developed secondary infections requiring euthanasia of the mouse prior to declines in body condition scores. In our experience, mice where the tumor ulcerated began to show clinical signs historically used as endpoints for these types of studies (e.g. hunched posture, decreased appetite, reluctance to ambulate) and the decision to euthanize could be made when these signs were seen. Body condition scoring did not supply additional information of use.

A third subcutaneous tumor study was tracked as part of this project. In this study, the tumor cells were injected subcutaneously over the flanks. The subsequent tumor growth effectively covered the regions that must be palpated to assess body condition score, rendering this technique ineffective. Subcutaneous tumor cell placement and its potential interference with accurate body condition scoring should also be considered.

The data from the animals on the intra-abdominal tumor study supported the initial hypothesis proposed by this grant. As predicted, terminal weight increased significantly as compared to
baseline in the animals treated with tumor cells, affirming that monitoring animals for weight loss as an endpoint would not be possible. This increase did not occur in the animals that were not treated with tumor cells. Likewise, the body condition score decreased significantly as compared to baseline in the animals treated with tumor cells, while the decrease did not occur in the animals that were not treated with tumor cells. This affirms the hypothesis that fat and muscle loss occurs on these studies, even when animal weight is increasing. The data also showed that when a score dropped one level, the animal exhibited increased chances of morbidity. However, we were unable to conclude that body condition score alone is useful used as an endpoint for these studies. We collected subjective data regarding animal posture and attitude, but were unable to analyze this data in comparison to the body condition score. We noted that animals that developed a score of 1.5 began to show hunched posture and lack of coordination prior to death, but the intra-observer interpretation of these criteria were too varied. Future studies will evaluate objective scales of assessment for these assessments and compare with the body condition score. We anticipate that further study will show that the body condition score proves a sensitive means of alerting the investigator and veterinary staff that death is approaching, allowing them to focus on the evaluation of other signs, such as hydration, posture, and appearance, when making the determination of appropriate time for euthanasia.

This study showed that body condition scores can be a beneficial addition to the health monitoring for animals that are used in studies with tumors that are intra-abdominal, such as ascites and other bulk producing tumors of the abdomen. We also confirmed that weight is not a useful parameter to track as a potential endpoint for these studies. We would also caution that our study confirms that weight loss as an endpoint should be used with care as the weight of the animal can fluctuate dramatically over a 12 hour period. This study also demonstrated that body condition score is not appropriate for all studies of tumor growth. The skin tumor growth studies that we monitored had no significant changes in body condition scores that were predictive of morbidity or mortality. More traditional clinical signs were found to be reliable for this model. This study confirms that body condition scores are a valuable adjunct to clinical health monitoring. Our future directions will develop an objective assessment of clinical signs such as posture and attitude, to facilitate timely intervention on these studies by providing additional criteria to use in evaluating the patient. Additional applications of body condition scoring should be considered in the future.

References: