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Feline malignant lymphoma: a multiway frequency analysis of a population

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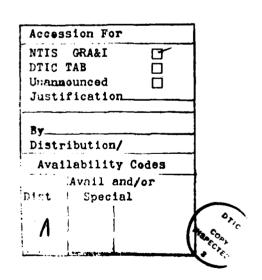
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SUMMARY

A multidimensional descriptive study of feline lymphoma was performed using age, sex, cell type, and tumor location of 1733 cases. The interactions between these 4 variables were determined and interpreted statistically. Particular consideration was given to the relationships of age to tumor location. age to sex, and tumor location to cell type, which were shown to be the major interactions in the study. The use of computer analysis of the multiway frequency table is discussed. The suggestion is offered that investigators consider the use of the multiway frequency table where possible in future studies of various diseases to enhance the depth of their conclusions.

Characteristics within a disease population can sometimes help to define the disease itself. These characteristics or variables rarely, if ever, exist without some mutual association to each other. Employing these associations or interactions as a foundation for a biological hypothesis, the total picture becomes more complete if one examines all interactions simultaneously. Incorporating many variables in a descriptive study tends to emulate nature more closely, but the issue of explaining the interactions becomes more difficult if too many variables are used. Since data in general have a quality of randomness, it follows that the more variables examined in a population study, the larger the sample size should be to assure adequate representation of those variables.

Malignant lymphoma is considered the most frequently occurring cancer in cats,[3,4] and is associated with a virus, feline leukemia virus (FeLV).[5-8]

The age-related incidence rate of the disease is bimodal, peaking at around 2 and 10 years.[9] Males have a 2-fold excess in incidence over females. Males peak in incidence at about 7 years of age. Females peak at about 1 year. According to some, malignant lymphoma has certain distinct cell types.[3,4,10] Others feel that the various types are manifestations of the same cell, but simply in different stages of maturation.[3,4,10] From the standpoint of tumor location, the internal form (visceral) is much more common in cats than the multicentric or solitary forms.[4]

A method of simultaneous examination of numerous discrete variables exists using a multiway frequency table.[1,2] The number of patients studied must be at least 10 times the number of cells in the table. This study presents such an analysis involving age groups, cell type, sex and tumor location of feline malignant lymphoma.

Materials and Methods

The study was conducted using the records of the Alameda/Contra Costa Animal Neoplasm Registry. A complete description of the registry is presented in the literature.[11] Tumor submissions were those first diagnosed between January 1970 and December 1978. A total of 1728 cases with complete information was submitted during that period. For this study, each case was categorized by sex (neutered female, intact female, neutered male, intact male); age group (0-4 years, 5-8 years, 9 years or older); tumor location (abdominal, thoracic, multicentric);[4] and cell type (lymphocytic well-differentiated, lymphocytic poorly-differentiated, histiocytic).[11] The age groups represent young, mid-age and old animals, respectively, and represent the two peaks and the trough of the bimodal age-related incidence.[9] The following codes were used to designate the variables: A-age, S-sex, C-cell type, and L-tumor location.

Cross-classification of the 1728 cases over the 4 variables created a multiway frequency table (Table 1). It consisted of 108 cells, 5 of which had zero frequency, probably due to sampling variations and would be expected to be non-zero if the samples were bigger. It is unlikely with the large sample size in this study that any empty cells would have a true expected value of zero. The zero frequencies were replaced by a frequency of one to facilitate computer analysis, thereby augmenting the sample size to 1733 cases.

Analysis of the multiway frequency was done using a program designated, BMDP3F.[1,2] This particular program tests for associations among the variables (bivariate and more complex) by fitting models involving the variables. The goal of this type of analysis is to find a model that has minimal complexity while exhibiting good fit, which is then identified as the "best" model. For each model fit, the program

prints a goodness of fit statistic, G^2, the log-likelihood ratio. This statistic, for large samples, is equivalent to the Pearson chi-square statistic. G^2 is calculated as:

$$G^2 = 2 \sum_{\text{cells}}^{\text{all}} \left[\text{observed} \times \log_e \frac{\text{observed}}{\text{expected}} \right]$$

A significantly large G statistic indicates a significant discrepancy between the observed frequencies and those expected for that model. The program has the capability of calculating the G^2 value for all models of increasing complexity starting from the complete independence model, (A)(S)(L)(C) in the present study, to the completely saturated model, (ASLC). As the models tested become more complex, the G^2 value becomes smaller, indicating a better fit. The changes in G^2 between adjacent models with corresponding changes in degrees of freedom are used to test whether increasing the complexity of the model achieves a significant improvement in the goodness of fit.

Where the number of possible models is large, a screening procedure is available to help focus on the several models that might be appropriate, while eliminating those models that warrant no consideration. The program automatically prints G^2 statistics for the complete independence model (A)(S)(L)(C) in the present study; the all possible first-order interactions model (AS)(AL)(AC)(SL)(SC)(LC); the all possible second order interactions model, (ASL)(ASC)(ALC)(SLC); and so forth if higher order interactions are possible. The "best" fitting model usually will be a model in complexity somewhere between the most complex model automatically fit indicating lack of fit and the least complex model automatically fit indicating good fit. It is between these two models that subsequent search efforts are directed. The search may be accomplished by fitting all models in complexity between these two models

or by using a stepwise selection procedure.' The forward stepwise
procedure starts with the least complex model automatically fit
indicating lack of fit and in a stepwise manner adds higher order
interactions until a good fitting model is achieved. At each step the
interaction added is that which produces the most significant reduction
in the size of the G^2 obtained for the model of the previous step. The
selection procedure stops at that step when the remaining interactions
cannot produce a statistically significant reduction in the size of G^2.
The backward elimination procedure starts with the most complex model
automatically fit indicating good fit and in a stepwise fashion removes
the highest order interactions until a model is fit that no longer
exhibits good fit. At each step the interaction removed is the
interaction that when removed makes the least impact on the size of the
resultant G^2. In the present study the screening procedure was used
with both the forward stepwise and backward elimination procedures.
Subsequently all possible models were fit as a check.

Results

The initial screening showed that the observed data were not compatible with the complete independence model and that the model containing all possible first-order interactions gave a good fit. Models involving second- and third-order interactions were excluded from further consideration. The best fitting model therefore was between (A)(S)(L)(C) and (AS)(AC)(AL)(SL)(SC)(LC).

A slight inconsistency emerged in the forward stepping procedure. The search went through 5 steps, yielding two models:

$$(AL)(AS)(LC)(SC)(AC) \qquad (1)$$

$$\text{and}$$

$$(AL)(AS)(LC)(AC)(SL) \qquad (2)$$

These two models were both indicated as suitable because the change in G^2 at step 5 between inclusion of (SC) or (SL) was very close.

Using the backward elimination procedure, the selection stopped after (SC) was removed, yielding model (2) above. In light of this uncertainty, it would be justifiable to use either model above, or the model with all 6 interactions:

$$(AL)(AS)(LC)(AC)(SL)(SC) \qquad (3)$$

In the forward stepping procedure, major reduction in G^2 at step 1 could have been made by including any of the interactions, (AL), (AS), or (LC) in the model. In fact, they were the first 3 interactions included in the model and represent the three strongest interactions. The remaining first-order interactions were of a lower magnitude as shown by their lesser impact on G^2 at step 1.

Since the final model contained nothing higher than the first order (bivariate) interactions, further statistical interpretation was possible by collapsing the 4-way table (Table 1) to a series of 2-way tables

representing each of the interactions (Tables 2-7). Each cross classification of Tables 2-7 contains 2 numbers. The upper number is the observed frequency for that cell. The lower number is the ratio of observed frequency to the expected frequency.

The chi square values for Tables 2, 3, and 4 were above 90 and represent the strongest bivariate reationships exhibited in the data. Table 2 describes the relationship between age and sex, and indicates that young intact cats of both sexes occur more frequently than expected in the sample. Additionally, fewer than expected intact animals of both sexes occurred in the mid-age and old-age groups. For neutered animals of both sexes the reverse age pattern was noted.

Table 3 compares age to tumor location. In young cats, there was a paucity of abdominal tumors and an abundance of thoracic tumors. In mid- and old-age cats, the abdominal-thoracic tumor pattern was reversed. The multicentric tumors showed little if any age predisposition.

In Table 4, the observed number of multicentric and abdominal tumors with the histiocytic cell type was higher than expected, while the well- and poorly-differentiated lymphocytic cell types in multicentric and abdominal locations were observed to be fewer than expected. Well- and poorly-differentiated lymphocytic thoracic tumors were abundant while histiocytic tumors of the thorax were sparse.

Although the chi square values for Tables 2-7 were all significant, those for Tables 2, 3 and 4 were considerably higher and the observed:expected ratios for Tables 2, 3 and 4 generally had a wider variation from a value of one. Nearly all the observed frequencies in Table 5 (cell type by sex group) were close to the expected frequencies. The exception to this was the paucity of histiocytic cell types among intact females and of lymphocytic well-differentiated among neutered

males, as well as a mild abundance of lymphocytic poorly-differentiated among intact females.

Table 6 is somewhat difficult to summarize. Suffice it to say that among young animals there was a paucity of the histiocytic cell type and that among the mid-age and old-age groups the histiocytic tumors were more abundant than expected.

In Table 7, it appears significant that the "female" row provided most of the departures from expected frequencies; that is, more thoracic and less abdominal and multicentric tumors than expected.

Discussion

Some of the reasons for conducting a study such as this are to define certain associations between disease characteristics that may suggest interactions of various biological phenomenon; or to point out a possible similarity with results of studies conducted from a different prospective, but arriving at the same point. Additionally, this study illustrates the use of the multiway frequency table analysis, a procedure not too often used in medical research.

A second-order interaction is one in which the pattern of association between 2 variables is dependent on the level of a third variable. One can perceive from this how a third-order interaction may behave. It is unfortunate that only first-order interactions emerged from this study, but the method of analysis is illustrated. Without this method, it would be tempting to conclude that trivariate and higher interactions do exist, based on only visual inspection of the tables. Such a conclusion would be done without the benefit of any statistical interpretation such as was attempted here.

Studies have shown that age plays a part in initiation of the disease.[13-16] Of the intact animals in our study, perhaps natural selection through lack of resistance eliminates them from the population at a young age, leaving fewer than expected in the older groups. On the other hand, since the reverse was true of neutered animals, perhaps sex hormones play a part in expression of the disease, once infected with the virus.

Similarity and possibly an explanation of the paucity of histiocytic cell types in thoracic tumors might be found in a study of human malignant lymphoma patients in Saudi Arabia[17] in which there was a tendency for histiocytic cell types to present as abdominal lesions. Another human study[18] indicates that a predominance of one B cell line is associated with well-differentiated lymphocytic cell type and cases with a decreased B cell population are primarily of the poorly-differentiated lymphocytic cell type. This same report suggests that future morphologic studies of the disease should include simultaneous immunologic classification. Using the multiway frequency table, one could expand that suggestion to include tumor location, which was shown to interact strongly with cell type in our study. Some say thoracic tumors are primarily T cells and abdominal tumors are B cells.[19-21]

Although this study was intended mainly to further characterize the disease, additional studies using the same techniques but with different variables may shed light on prognosis and therapy of the disease.

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TABLE 1—Multiway frequency table of 1733 cases of feline lymphosarcoma grouped by age, sex cell type and tumor site

Location	Cell	Sex	No. of cases by age		
			Young	Middle	Old
Multi-Centric	Well	NF	30	6	10
		F	13	4	2
		NM	28	13	7
		M	32	2	2
	Hist	NF	31	13	17
		F	26	4	4
		NM	70	36	31
		M	19	11	14
	Poor	NF	17	8	9
		F	19	5	1
		NM	28	16	12
		M	25	8	2
Abdominal	Well	NF	17	7	12
		F	11	2	6
		NM	23	19	18
		M	23	3	4
	Hist	NF	28	22	31
		F	27	6	8
		NM	67	57	36
		M	44	21	16
	Poor	NF	18	8	12
		F	25	2	8
		NM	40	17	17
		M	30	5	4

Thoracic	Well	NF	25	2	2
		F	38	(0)	1
		NM	34	6	2
		M	32	4	1
	Hist	NF	16	3	5
		F	15	2	(0)
		NM	21	8	2
		M	20	(0)	(0)
	Poor	NF	31	3	2
		F	28	6	(0)
		NM	36	13	3
		M	17	9	2

Well = Lymphocytic, well-differentiated; Hist = histiocytic;

Poor = lymphocytic, poorly-differentiated.

NF = neutered female, F = female, NM = neutered male, M = male.

(0) = assigned value of 1 for computation purposes.

TABLE 2—Bivariate relationship of age to sex (AS)

| Age | | Sex | | | |
---	---	Intact male	Neutered male	Intact female	Neutered female
Young	Observed	182	347	207	233
	Ratio	1.172	0.840	1.238	0.940
Mid-age	Observed	62	195	32	72
	Ratio	0.763	1.397	0.567	0.360
Old	Observed	46	128	32	97
	Ratio	0.675	1.093	0.675	1.380

Chi square = 40.55 with 6 d.f. p < 0.001.

Ratio: Observed frequency/expected frequency.

TABLE 3—Bivariate relationship of age to tumor location (AL)

Location		Age		
		Young	Mid-age	Old
Multicentric	Observed	393	136	108
	Ratio[*]	1.000	1.025	.970
Abdominal	Observed	353	169	172
	Ratio	0.824	1.169	1.418
Thoracic	Observed	323	56	23
	Ratio	1.303	0.669	0.327

Chi square = 102.339 with 4 d.f.   p < 0.001.

[*] Ratio: Observed frequency/expected frequency.

TABLE 4—Bivariate relationship of tumor location to cell type (LC)

Cell type		Tumor location		
		Multicentric	Abdominal	Thoracic
Lymphocytic,	Observed	154	145	148
well-differentiated	Ratio*	0.937	0.810	1.427
Histiocytic	Observed	326	363	95
	Ratio	1.131	1.156	0.522
Lymphocytic, poorly-	Observed	157	186	159
differentiated	Ratio	0.851	0.925	1.365

Chi square = 109.93 with 4 d.f. p < 0.001.

* Ratio: Observed frequency/expected frequency.

TABLE 5—Bivariate relationship of sex to cell type (SC)

Cell type		Sex			
		Intact Male	Neutered male	Intact Female	Neutered female
Lymphocytic, well-	Observed	103	150	83	111
differentiated	Ratio*	1.024	0.868	1.187	1.070
Histiocytic	Observed	177	328	93	186
	Ratio	1.003	1.082	0.759	1.023
Lymphocytic, poorly-	Observed	110	192	95	105
differentiated	Ratio	0.974	0.989	1.210	0.902

Chi square = 20.0206 with 6 d.f. p < 0.005.

* Ratio: Observed frequency/expected frequency.

TABLE 6—Bivariate relationship of age to cell type (AC)

Cell type		Age		
		Young	Mid-age	Old
Lymphocytic, well-	Observed	311	69	67
differentiated	Ratio*	1.128	0.741	0.357
Histiocytic	Observed	434	184	166
	Ratio	0.897	1.127	1.211
Lymphocytic, poorly-	Observed	324	108	70
differentiated	Ratio	1.046	1.033	0.798

Chi square = 30.53 with 4 d.f. p < 0.001.

* Ratio: Observed frequency/expected frequency.

TABLE 7—Bivariate relationship of sex to tumor location (SL)

Location		Sex			
		Intact male	Neutered male	Intact female	Neutered female
Multicentric	Observed	145	251	83	158
	Ratio[*]	1.011	1.019	0.833	1.069
Abdominal	Observed	150	294	95	155
	Ratio	0.960	1.096	0.875	0.963
Thoracic	Observed	95	125	93	89
	Ratio	1.050	0.804	1.479	0.954

Chi square = 29.0236 with 6 d.f. $p < 0.001$.

[*] Ratio: Observed frequency/expected frequency.

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