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Editorial

The biology of haematogenous metastasis in human uveal malignant melanoma

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Abstract. Using data on 4726 cases of uveal melanoma collected by the Registry, of Ophthalmic Pathology at the Armed Forces Institute of Pathology, my co-workers and I have been studying the biology of haematogenous metastasis. We have developed methods for evaluating separately the relationships between prognostic factors and cure-rate and median survival time for uncured patients. We have discovered that size of the tumour and size of the largest nucleoli in the cells of the uveal melanomas related equally to cure-rate and median survival time. The presence of Callender's epitheloid-type cells in the tumour and increased patient age had little effect on cure-rate but dramatically reduced the median survival time.

Key words: Uveal malignant melanoma – Cure-rate – Survival time – Haematogenous metastasis

The Ophthalmic Pathology Department of the Armed Forces Institute of Pathology has been accumulating a database of cases of uveal melanoma, the most common primary intraocular tumour, since the formation of the Registry of Ophthalmic Pathology in 1931. In each case a concerted effort has been made to obtain follow-up data and there now exists a database of 4726 cases with long-term follow-up information. Using cases from the Registry of Ophthalmic Pathology, Wilder and Callender (1939) and Wilder and Paul (1951) were able to demonstrate a relationship between cytological features of uveal melanomas and death of the patients due to metastatic disease. Callender (1931) classified uveal melanoma cells into two main types: spindle and epithelioid. Tumours that contain epithelioid cells are associated with a worse outcome. The biological mechanisms responsible for this association are unknown.

Uveal malignant melanoma provides an excellent model for the study of haematogenous metastasis. The eye lacks lymphatic vessels and, therefore, malignant tumours confined to the eye can only metastasize via the haematogenous route. Uveal melanoma cells once they gain access to the blood stream appear to home to the liver. In 80% of cases of uveal melanoma, the initial site of metastases is the liver and in 90% of the cases the liver is involved prior to death. The ability of uveal melanoma to metastasize haematogenously, while still confined to the eye, must be in part a characteristic of epithelioid-type uveal melanoma cells. Retinoblastoma, the second most common primary intraocular tumour, usually does not metastasize until after it has invaded the orbit. From the orbit it gains access to lymphatic vessels located in the anterior orbit and conjunctiva. Unlike uveal melanoma, the inial metastases from retinoblastoma are often to regional lymph nodes.

The process of haematogenous metastasis is a sequential one involving multiple steps. These include growth and local invasion in the primary site, neovascularization, vascular invasion, embolization, transportation to the metastatic site, arrest at the metastatic site, extravasation at the metastatic site, initial growth and neovascularization, growth of the tumour cells in the established metastasis and re-metastasis (Fidler 1990). In a sequential process each step must be completed before the next one can begin; therefore, if the metastatic process is interrupted at any point, the patient is cured. For patients with uveal melanoma, as with many cancers that occur in adults, there is no effective therapy once there is established metastases. For these reasons, enucleation of an eye containing a malignant melanoma is curative only if it occurs before neoplastic cells, capable of forming a metastasis, have disseminated. Thus, factors that affect the probability that a patient will be cured should relate to the ability of the tumour cells to invade blood vessel walls and establish a metastasis.

Survival time for uncured patients is a function of how rapidly the metastatic process is completed. Because survival time is usually measured from the time of treatment to the time of death and since uncured patients must have disseminated tumour cells prior to, or at the time of, treatment, survival time is mainly a function of events that take place after establishment of the me-

tastasis. From these premises, it should be apparent that the biological factors that affect cure are different for those that affect survival time.

In addition to growth rate of the tumour cells, survival time is a function of lead time and maximum tumour burden. Lead time is the time interval between metastasis and treatment. One would expect that larger tumours would have a longer lead time and a shorter survival time. However, if lead time were to account for a significant portion of the time from metastasis to death, then examination of patients prior to surgery should frequently disclose signs of metastatic disease. Since metastases are rarely discovered prior to enucleation for uveal melanoma (Zimmerman and McLean 1980), lead time should not be a major factor with this tumour.

Patient to patient variation in the size of the tumour burden that is tolerated should also not have a major effect on the survival time. Because of exponential growth, a difference of one or two doubling times has a large effect on the size of the tumour burden but a small effect on the life span of a tumour that is approximately 40 doubling times long. Thus, survival time for patients dying with haematogenous metastasis of uveal melanoma is primarily a function of the growth rate of the tumour cells.

Uveal melanoma, like most neoplasms that occur in adults, can kill the patient many years after excision of the primary tumour. To correct for the effect of intercurrent disease, survival analysis of uveal melanoma is usually performed using a single decrement life table (Elandt-Johnson and Johnson 1980) or adjusted actuarial method (Cutler and Axtell 1969) to estimate the waiting time distribution for uveal melanoma related deaths. In constructing this adjusted life table, deaths due to causes unrelated to uveal melanoma are coded as withdrawn alive at the time of death. We have used the term "cause specific survival distribution" for the waiting time function to emphasize that this survival distribution provides an estimate of the survival function that would

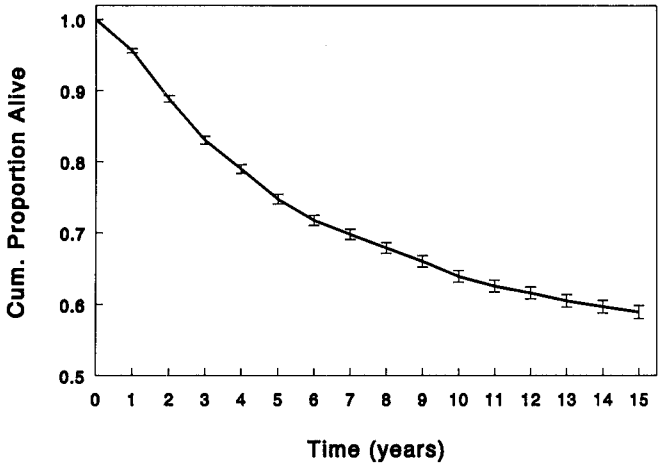

Fig. 1. Cause specific survival function (±standard error) estimated for 4726 patients with uveal melanoma by coding deaths from causes unrelated to the uveal melanoma as withdrawn alive at the time of their death. Time zero is the time of enucleation

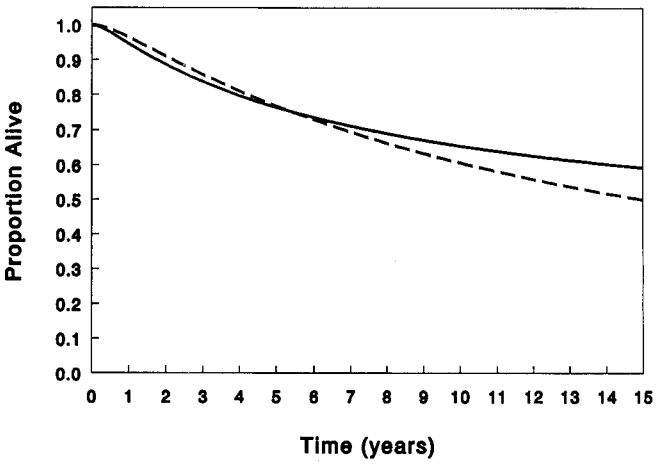

Fig. 2. Hypothetical cause specific survival functions with log normally distributed survival times. Comparison of a population with a median survival time of 7.5 years and a 40% cure rate (*solid curve*) with a population having a median survival time of 15 years and a 0% cure rate (*dashed curve*)

be obtained if causes of death unrelated to uveal melanoma were eliminated (Fig. 1). If some of the patients in the study are cured, then the limit of the cause specific survival function with increasing time is not zero but approaches asymptotically a value that provides an estimate of the cure-rate. The rate at which the asymptote is reached is a function of the survival times of the uncured patients.

For cancers with slowly growing metastases, such a uveal melanoma, even with long-term follow-up data it may be difficult to estimate graphically the cure-rate from the asymptote of the cause specific survival function (Fig. 1). This problem can be illustrated by comparing two hypothetical populations with log normally distributed survival times. Let the first group have a 40% cure rate and a median survival time of 7.5 years (solid curve, Fig. 2) and the second group a 0% cure-rate and a 15 year median survival time (dashed curve, Fig. 2). During the first 10 years after enucleation, there is little difference between these two survival curves. In fact, for moderate-sized populations, traditional statistical tests (Mantel and Henzel 1959; Cox 1972), which are especially sensitive to consistent differences is survival rates, would not detect a statistical significant difference in survival. Despite the small differences in 5 or 10 year survival rates, there is a major difference in biological mechanisms implied in the formulation of these two hypothetical populations. To achieve our goal of studying the biology of cancer in humans, we needed a reliable method for determining from survival data the cure-rate and median survival time.

Boag (1949) suggested that the cure-rate and median survival time can be estimated using the maximum likelihood method, if the cause specific survival distribution can be mathematically defined. For many cancers, including carcinoma of the cervix and uterus (Mould and Boag 1975), head and neck cancers (Mould et al. 1976) and breast carcinoma (Rutqvist et al. 1984) the log normal function provides a good fit to the cause specific

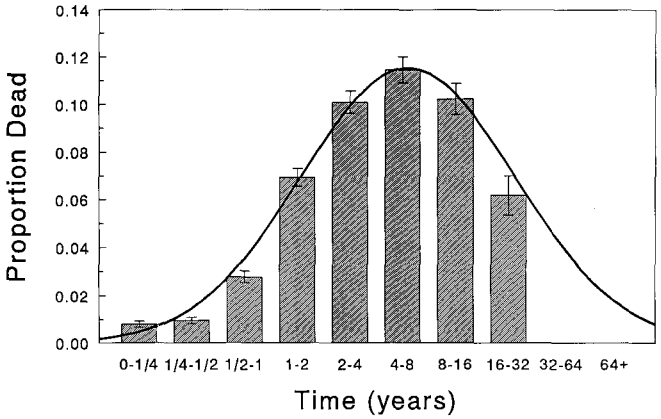

Fig. 3. Density function of related deaths for 4726 patients with uveal melanoma. Logarithmic time scale and approximate normal distribution truncated at 32 years of follow-up after enucleation (bar graph, ±standard error). Solid curve is the maximum likelihood estimate of the log normal function (cure-rate = 0.42, median survival time = 6.5 years and standard deviation = 1.4 log-years)

survival distribution and we have found that uveal melanoma conforms to this model (Fig. 3). Using a log normal survival model, we have found that the proportion cured for 4726 patients with uveal melanoma was 0.42 and the median survival time was 6.5 years. The standard deviation of the log normal survival distribution was 1.4 log-years. This log normal function is illustrated by the solid curve in Fig. 3. We have extended Boag's model to express the proportion cured and the median survival time as multivariate functions of features of uveal melanomas and host factors (Gamel et al. 1990).

The first variable we studied using our extension of Boag's log normal model was tumour size (Gamel et al. 1990). We have found that the most prognostically significant way to measure the tumours was by simply considering the largest dimension. Measuring maximum and minimum diameters or base and height did not improve the prognostic accuracy. We found that larger tumours were associated with a much lower probability of cure and a much shorter median survival time. Tumours measuring 8 mm had a 60% cure-rate, whereas 20 mm tumours had an 18% cure-rate. Patients with tumours measuring 8 mm had a median survival time of 12 years. With 20 mm tumours, the patient's survival time decreased to 3 years. We believe that this 9 year difference is too large to be explained on the basis of lead time and; therefore, we conclude that larger tumours produce more rapidly growing metastases. The effect of size on both cure and survival time suggests that as uveal melanomas enlarge they produce new mutations resulting in a more aggressive phenotype.

We have been particularly interested in determining what features of uveal melanoma are most responible for the relationship between Callender's classification and survival of patients with uveal melanoma. We have used morphometry to measure a number of the cytological features of uveal melanoma cells (Gamel et al. 1982). The feature that we have found that best predicts patient outcome is the size of the largest nucleoli in the tumour.

Table 1. Multivariate relationship (t-value) between five prognostic variables and the proportion cured and the median survival time (data from Gamel et al. 1993). Negative t-values indicate a negative relationship

Prognostic variable	Proportion cured	Survival time
Tumour size	−7.34	−7.26
Nucleolar size	−5.54	−5.11
Cell type	−2.85	−9.17
Patient's age	0.55	−4.97
Patient's sex	−2.38	1.49

We have developed an algorithm, which we have used to calculate the mean of the diameters of the ten largest nucleoli (MLN) encountered in a strip 5 mm long by one 100X oil immersion field wide through the centre of the tumours (McCurdy et al. 1991). We found a strong relationship between MLN and survival of the patients. The larger the nucleoli the lower the cure-rate and the shorter the median survival time. Our multivariate analysis (Table 1), indicates that size of tumour and MLN are both significant predictors of cure and survival time. Thus, if the poor prognosis associated with larger tumours is due to the content of more malignant cells then not all of this association is expressed in the measurement of MLN.

The behaviour of a multivariate model that contained both MLN and Callender's cell type (Table 1) provides information on the relationship between these variables in determining survival. Callender's classification was used to divide the tumours into two groups, those with and without epithelioid cells. In this model MLN was strongly associated with both cure and survival time but Callender's cell type was only weakly associated with cured fraction. Surprisingly, the association between cell type and survival time was the most highly significant of any of the relationships that we studied (Table 1). This indicated that most of the prognostic information in the Callender classification that relates to cure is included in MLN but there is considerable additional information in cell type relating to survival time. Thus, features of uveal melanoma cells other than nucleolar size used in the subjective classification of uveal melanoma cells as epithelioid type must be strongly related to the growth-rate of the tumour cells.

The observed effect of patient's age in the log normal model was unexpected. With increasing age there was a marked reduction in the median survival time but no effect on the probability of cure (Table 1). The poorer prognosis associated with increasing patient age observed with many tumours is thought to be related to a decrease in immune surveillance. If this is true, then these age related immune mechanisms must be more effective in slowing the growth-rate of uveal melanoma cells than preventing them from establishing metastases.

Hopefully, this work will encourage investigators to study other tumours using a survival model that permits the separate analysis of the effects of prognostic variables on cure-rate and survival time. Rutqvist et al. (1984) have performed such an analysis. They studied

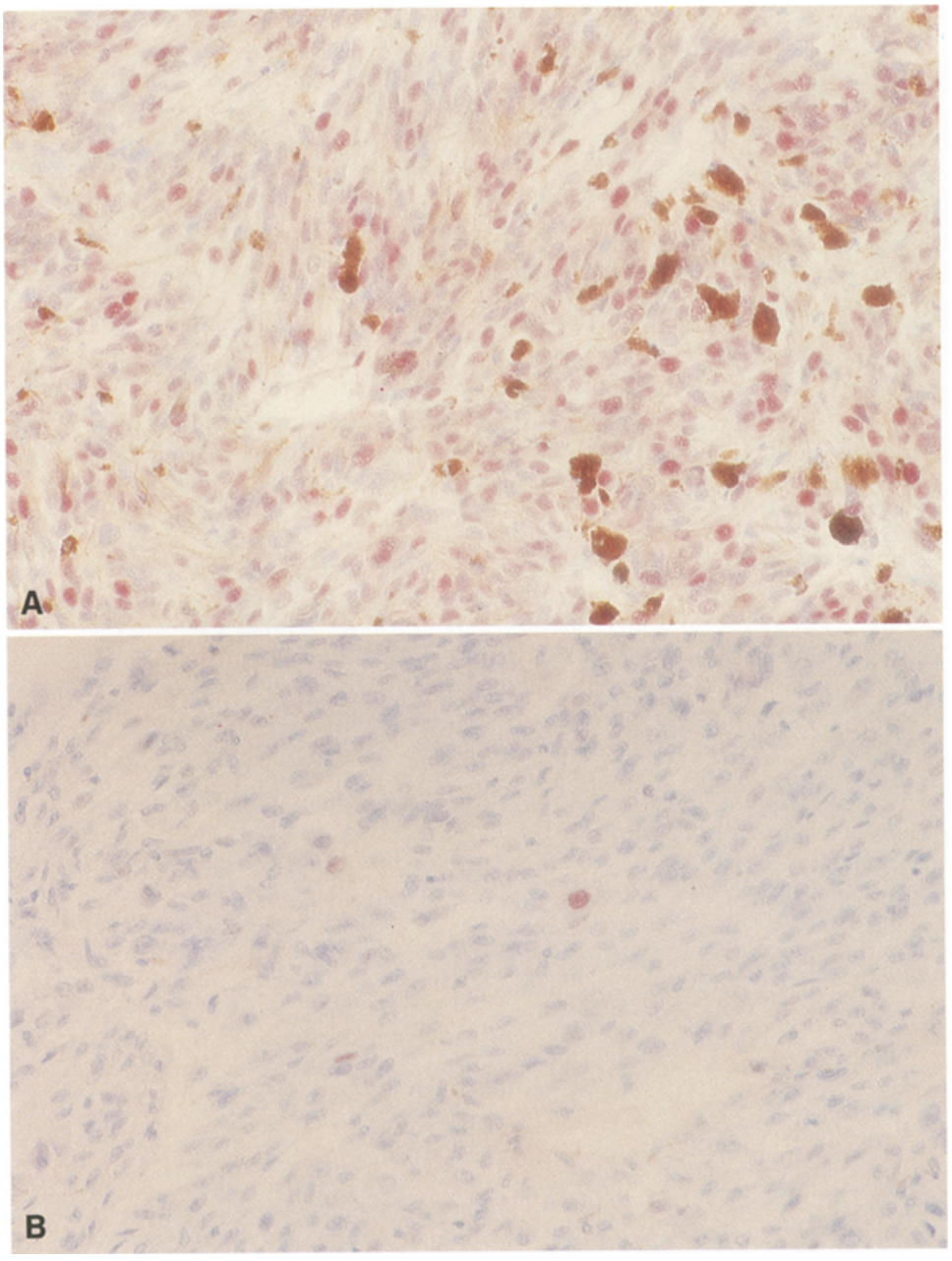

Fig. 4. Proliferating cell nuclear antigen (PCNA) immunoreactivity in uveal malignant melanomas. **A** Tumour in which the majority of the neoplastic cells express PCNA (red aminoethylcarbazole reaction product). **B** Tumour with low expression of PCNA. In both tumours nuclei containing PCNA tend to be plumper (more epithelioid)

the effects of clinical stage and patient age, in women with breast cancer. Advanced stage decreased both the cure-rate and the survival time; however, increased age decreased the cure-rate but it had no effect on median survival time. This observation was the opposite of what we observed with uveal melanoma. The different effects of age in patients with uveal melanoma and breast carcinoma suggest that the mechanisms responsible for these associations are very different in these two tumours. Rutqvist et al. (1984) did not analyse the histological types of the breast carcinomas in their study and the asssociation between age and cure-rate could be due to this or other confounding effects.

The use of the log normal model to study the biology of metastasis of uveal melanomas can be extended to the molecular level using immunohistochemistry. Antibodies are being developed that are reactive against proteins that are believed to be involved in the processes of invasion and proliferation of neoplasms. Two such proteins that can be detected immunohistochemically in paraffin-embedded, formalin-fixed tissue are urokinase plasminogen activator (u-PA) and proliferating cell nuclear antigen (PCNA). Quax et al. (1991) have shown that human cutaneous melanoma cell lines that produce u-PA have the highest frequency of spontaneous lung metastasis following subcutaneous inoculation. PCNA is a DNA-δ-polymerase that is strongly expressed during S-phase of the cell cycle. Our preliminary studies indicate a broad range of PCNA activity in uveal melanomas (Fig. 4).

We would also like to use the log normal model to investigate the effect of tumour infiltrating lymphocytes

(TIL) on cure-rate and survival time. In a previous study (Cruz et al. 1990), we found that the presence of TIL in uveal melanomas was associated with a lower survival-rate. This finding is the opposite of what is observed with most solid tumours in adults, where the presence of TIL conveys a more favourable outcome. We hypothesize that the different behaviour associated with TIL in uveal melanomas may be related to the lack of lymphatic drainage from the eye. This is known to confer immune privilege to the eye, but in addition it may make it more difficult for uveal melanomas to disseminate tumour cells than most cancer. Thus, if dissemination is a risk factor for metastasis and dissemination of tumour cells is required for uveal melanomas to invoke the immune response that is manifested by the infiltration of lymphocytes then the presence of TIL should be associated with a lower cure-rate. However, the presence of TIL may have no effect or possibly a beneficial effect on survival time.

In conclusion, I have discussed a method that we are using to analyse separately cure-rate and survival time in the study of the biology of uveal melanoma in humans. The existence of factors that differentially affect cure rate and survival time (Table 1) emphasizes the value of this methodology, which should be applicable to other cancers.

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