Photoreceptor differentiation in retinoblastomas and its significance in prognosis

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SUMMARY Fleurette is an expression of photoreceptor differentiation and its presence is said to indicate a good prognosis. Twenty-four eyes from 22 cases of retinoblastoma sent to the Mitrani Foundation laboratory between the years 1964 and 1976 were reviewed histologically and clinically. The presence of differentiation in the tumour was not always associated with a good prognosis. The most important prognostic factor seemed to be the length of time the tumour is allowed to remain in the eye. The longer it remains the more undifferentiated the tumour cells become and the greater the probability that it will spread into the choroid and the optic nerve and out of the eye through the sclera and metastasise throughout the body. The presence of gliosis seems to improve the prognosis.

The prognosis of retinoblastomas has been thought to be related to the amount of differentiation of the tumour cells (Reese, 1963; Brown, 1966). The signs of differentiation taken into consideration have been the formation of fibrils and rosettes (Reese, 1963; Brown, 1966). Taktikos (1966) claimed that 'no significant relationship exists between the malignancy of retinoblastomas and the degree of their histological differentiation, which is unrelated to the mortality rate, the length of survival and the incidence of fatal complications'.

There is one point, however, on which both sides seem to agree—that is, that early diagnosis and prompt and appropriate treatment is the most important factor in the prognosis of the retinoblastomas (Taktikos, 1966).

Brown (1966) reported that a retinoblastoma first forms true rosettes which with time decrease in number and change into pseudorosettes or into anaplastic cells. Herm and Heath (1956) and Carbajal (1958) suggested that the longer the retinoblastoma is present the more undifferentiated it becomes. The conclusion is that it is not the degree of differentiation of the tumour cells but the length of time the tumour has been in the eye that counts in predicting the prognosis. In 1969 the fleurette, an expression of photoreceptor differentiation of the retinoblastoma cells was described for the first time (Ts'o *et al.*, 1969). This revived the question of the importance

of histological differentiation in prognosis. While Ts'o *et al.* (1969) and Yanoff and Fine (1975) believe that the presence of fleurettes confers a better prognosis, Sevel *et al.* (1974) claim that it has no prognostic value.

To come to a better understanding of this controversial problem we reviewed our cases of retinoblastoma histologically and clinically.

Material and methods

Twenty-four eyes from 22 cases of retinoblastoma were sent to the Mitrani Foundation Eye Pathology Laboratory between 1964 and 1976. We have reviewed these histologically and clinically. At least five slides from each eye were histologically examined under high magnification (immersion). The whole surface of the tumour was covered in the examination. Stains used in addition to the routine haematoxylin and eosin included Masson's trichrome, PAS, and PTAH (phosphotungstic acid haematoxylin). We noted the type of tumour (retinoblastoma or neuroepithelioma); the kind and degree of differentiation of the cells, with special emphasis on the presence of fleurettes and glial cells; and the presence of calcification and necrosis, spread of the tumour out of the sclera into the choroid, optic disc, optic nerve, or anterior chamber. We recorded the ages at which the child was first examined and the tumour diagnosed; the age at which enucleation was performed; the occurrence

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Fig. 1 Rosette made up of undifferentiated cells. $\times 1400$

of scleral perforation during enucleation; the uni- or bilaterality of the tumour; and the familial incidence.

Results

HISTOLOGY

The tumour in all the 24 eyes was a retinoblastoma. We could see a few rosettes in 14 of them but not enough to merit calling the tumour a neuroepithel-

ioma. In three cases there were those clear areas that Ts'o *et al.* (1969) considered characteristic of the presence of fleurettes. In all these areas we found fleurettes in different degrees of differentiation.

In general, the type of tumour cells varied from the very anaplastic type to the more or less differentiated photoreceptor or glial cell. Photoreceptor-like cells were seen mostly in true rosettes or around them. Rosettes were formed either by cells with little cytoplasm and their nucleus near the limiting

Fig. 2 Rosette made up of partially differentiated tumour cells. $\times 1400$

Fig. 3 Rosette with one photoreceptor cell (arrow). $\times 1400$

membrane of the central lumen (Fig. 1), by cells with a relatively abundant cytoplasm not protruding inwards into the lumen (Fig. 2), or by cells showing cytoplasmic extensions into the lumen (Fig. 3). Incomplete rosettes were formed by rows of three to four cells parallel with each other, with a clear photoreceptor differentiation. Rosettes with all their cells showing photoreceptor differentiation were also present (Fig. 4). These rosettes showed no clear-cut limiting membrane and lumen centrally.

There were photoreceptor-like cells arranged around a central line. These last three formations of cells showing an advanced degree of photoreceptor differentiation could be called fleurettes. In addition we saw single photoreceptors among anaplastic cells (Fig. 5). Glial differentiation expressed itself as fusiform cells with long bipolar extensions arranged parallel with each other and as fibrillar areas around vessels. There were also fibrillar perivascular structures diagnosed as fibrosis with the Masson's

Fig. 4 Rosette with all cells presenting photoreceptor differentiation (Fleurette) (arrow). $\times 1400$

Fig. 5 *Single photoreceptor (arrow). ×1400*

trichrome stain. Numerous vessels had thickened walls or obstructed lumens (Fig. 6). There were large areas of necrosis and calcification.

Histology of irradiated tumours

Two of the 24 eyes were irradiated before enucleation. The tumour and the retina in each showed large areas of destruction, rosettes, loosening of cell connections and anaplastic retinoblastoma cells, and dispersion.

CLINICAL SURVEY

Seven of our 22 patients died and 15 are still alive.

In eight cases the child was first examined between 1 month and 1 year of age and in 14 cases between 1 and 5 years. In the first group three out of eight died one, 2½, and four years respectively after enucleation. In the second group four out of 14 died during the first year after enucleation.

Usually eyes were enucleated one to two weeks after diagnosis. In five cases it was delayed for from

Fig. 6 *Occluded capillary in tumour. ×1400*

four months to four years after diagnosis. Four of these were unilateral cases and one was bilateral. All of them died in the first year after enucleation. Enucleation in those children whose retinoblastoma was diagnosed at the age of 1 to 4 years and died (four out of 14) had also been delayed.

Three of our 22 cases (24 eyes) were bilateral and in two of them we could examine both eyes. The remaining 19 cases were unilateral. In one bilateral case the patient died three years after the second enucleation. The patient in another bilateral case is still alive eight months after the second enucleation.

One case of bilateral retinoblastoma in which only one eye was enucleated and the second treated conservatively, is still alive six months after enucleation. In six out of the 19 unilateral cases the patient died 1½ to four years after enucleation. The follow-up of the remaining 13 cases extends from four months to four years.

Scleral perforation occurred in three cases. One of the patients died 2½ years after enucleation and the other two patients are still alive 10 years after enucleation.

No familial incidence was noted in our series of cases.

HISTOLOGY RELATED TO PROGNOSIS

Presence of rosettes Rosettes were seen in 14 out of the 24 eyes. They were present in four out of the seven cases in which the patient died.

Presence of fleurettes Fleurettes were seen in three cases. The patient in one case, a unilateral retinoblastoma, is still living 2½ years after enucleation. The patient in one case of a bilateral tumour died four years after enucleation. The patient in another bilateral case is still alive but follow-up has been for only eight months.

Presence of gliosis Gliosis was seen in three cases. The patients in all of them are alive eight months to eight years after enucleation.

Tumour spread outside the sclera The tumour had spread out of the sclera in six eyes pertaining to five cases. In four of them enucleation was delayed because the parents refused permission. All the patients died. The fifth case, a bilateral one, was diagnosed at the age of 1 month in both eyes. The first eye was operated soon after and the second 1½ years later after irradiation had failed to check the growth of the tumour. The child in this case is still alive but so far follow-up has not exceeded eight months.

Spread into the choroid This was observed in 12 eyes and 10 cases. In all of the seven patients who

died the tumour had extended into the choroid. In the three who are still alive follow-up has not been for more than one year.

Spread into optic disc and optic nerve Spread only into the optic disc was seen in one case, and the patient is still alive one year after enucleation. Spread into the optic nerve was seen in 12 eyes and 11 cases. In all seven of the children who died the tumour extended into the optic nerve. The patients in the four remaining cases are alive and have been followed up for from eight months to three years.

Calcification and necrosis These were seen in all cases and were unrelated to prognosis.

Discussion

The term fleurette is applied to an elaborate form of photoreceptor differentiation of retinoblastoma cells. Some research workers think that the presence of fleurettes implies a better prognosis. Ts'o *et al.* (1969) reported no mortality in a series of eight cases of retinoblastoma with fleurettes followed up for eight years. Sevel *et al.* (1974), however, claimed that single photoreceptor cells or fleurettes were of no prognostic significance. We found the presence of fleurettes is not always associated with a good prognosis.

Fleurettes and well-differentiated single photoreceptors were seen in three cases. The patient in the unilateral case is still alive. Of the two patients with bilateral disease one is already dead and the other one, who has had both eyes enucleated and in whom both tumours were invading the sclera, choroid, and optic nerve, has been followed up for only eight months and is in a bad general condition. Interestingly, in these two bilateral cases fleurettes were seen only in the first enucleated eye and not the second, which was enucleated after unsuccessful irradiation.

The importance of rosettes in determining prognosis has been already evaluated. Tsukahara (1960) found no significantly greater mortality rate in patients with less differentiated tumours than in those with the well differentiated type. Reese (1963) ascribed a good prognosis to their presence. Our cases with rosette formation were too few in number to draw any conclusion.

The factor which seems most to affect prognosis is the length of time the tumour is allowed to stay in the eye—the longer the worse the prognosis. When seeing a child for the first time we cannot know how long the tumour has been present unless the patient is only a few weeks old. We may suppose, however, that the older the child the longer the

tumour has been present. To this we could attribute the fact that the four out of the 14 of our children who had been examined for the first time when between 1 and 4 years of age died within one year after enucleation. But in these children enucleation was also delayed and this is a more likely reason for the bad prognosis. Indeed, in all five of our cases in which enucleation was delayed died soon.

The worsening of the prognosis owing to prolonged presence of the tumour may be perhaps because retinoblastoma cells become more and more undifferentiated with time (Brown, 1966). But accordingly our observations suggest that it is due mostly to the fact that as time goes on the tumour more likely to invade the sclera, the choroid, or the optic nerve. Spread of the tumour into the choroid and optic nerve caused general metastases in the body, which conservative treatment (irradiation and cytostatics) failed to check in five of our fatal cases. The only effect of the conservative treatment was to prolong the life of the child; it could not prevent a fatal outcome. We are unable to say whether tumour invasion of the anterior chamber had any prognostic significance in our cases.

The presence of gliosis might be meaningful. Indeed, our three cases who had gliosis are all alive. Gliosis is a sign of differentiation, and its presence might improve the prognosis. However, three cases is too few a number from which to draw any definite conclusion. Moreover, in none of them was there any extraocular spread of the tumour.

Unilaterality or bilaterality did not seem to be relevant to prognosis in our cases.

Scleral perforation during surgery is a rare complication but it might worsen the prognosis.

Although the small number of our cases does not allow us to draw such a conclusion, we contend that scleral perforation during surgery disseminated tumour cells in the orbit and therefore adversely affected the prognosis.

In conclusion, we can say that photoreceptor differentiation in retinoblastomas does not mean a better prognosis. Rather it is the length of time that the retinoblastoma remains in the eye untreated, or inadequately treated, and extraocular spread of the retinoblastoma through the sclera and into the choroid and optic nerve that makes for a poor prognosis. It is two factors and not the presence of photoreceptor differentiation that must be considered in giving a prognosis.

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