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Long-term follow-up of germinoma after stereotactic biopsy and brain radiotherapy: a cell kinetics study

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Abstract The primary aim of this study is to report the long-term outcome of pineal and suprasellar germinoma after stereotactic biopsy and whole brain radiotherapy. The second purpose is to report an investigation of the biological features and cell kinetics of this peculiar and enigmatic brain tumour. Of 34 supratentorial germ cell tumours diagnosed and treated between 1980 and 1993, 20 patients were found to be affected by true germinoma localized in the pineal and/or suprasellar regions. The diagnosis was achieved by stereotactic biopsy in all cases. In 14 patients, the potential proliferative activity of the tumour was investigated by (^{3}H)thymidine in vitro binding and labelling index determination. Chorionic gonadotropin, α-fetoprotein and embryonal carcinoma antigen were negative in the cerebrospinal fluid of these patients. All but 1 patient underwent whole brain radiotherapy. Clinical and neuroradiological follow-up ranged between 3 and 13 years (mean 8). Complete clinical and neuroradiological recovery was achieved in all patients after treatment. Fatal recurrences owing to neuraxis dissemination occurred in three cases. The labelling index in the whole series ranged between 0.1 and 5% (median 2.5). Only syncytiotrophoblastic cells had proliferative activity, while none of the lymphoid-like cells showed thymidine labelling.

Key words Intracranial germinoma · Cerebrospinal fluid dissemination · Stereotactic biopsy · Radiotherapy

Introduction

Intracranial germinoma is a rare tumour mainly affecting the pineal and suprasellar regions. In the last 2 decades, two diagnostic and therapeutic strategies have divided neurosurgeons and radiotherapists: stereotactic biopsy or direct surgery to assess the histological diagnosis followed by whole brain or craniospinal radiotherapy [3, 12, 22, 23] is the strategy used by neurosurgeons. The second strategy, preferred by radiotherapists, includes "soft" preliminary radiotherapy to assess the radiosensitivity of the neoplasm and to confirm the presumptive neuroradiological diagnosis before definitive radiotherapy is performed [19, 30]. Nevertheless, general agreement exists that supratentorial germinomas may definitely be cured. The mass disappearance shown by computed tomography (CT) and magnetic resonance imaging (MRI) occurs after treatment [5, 6, 10, 13, 15, 18, 21, 26–29]. In spite of these considerations, some patients suffer dramatic fatal recurrences, even many years after the diagnosis. This late development is poorly recognized in the literature. One of the aims of this study is to assess the long-term prognosis in a large series of patients. The conventional histological diagnosis in this series has been reinforced by a cell kinetics study to search for further biological features of these rare enigmatic brain tumours.

Materials and methods

Between 1980 and 1993, the histological diagnosis of germinoma was made in 20 patients (16 male) aged 3–42 years (mean 20). Fifteen cases had a single lesion in the pineal region; 2 had a single lesion in the suprasellar region; 1 had ectopic localization in the frontal white matter. Two cases had localizations involving both the pineal and suprasellar regions. CT and magnetic resonance (MR) features included homogeneous contrast enhancement in all cases and the presence of small areas of calcification in 11 patients. Obstructive hydrocephalus occurred in 11 patients in whom the tumour was localized within the pineal region. These patients underwent shunting procedures (in 5 patients the shunt was implanted before the correct histological diagnosis; in 4 patients shunting was performed at the time of stereotactic biopsy; the other 2 cases had a shunt implanted after the stereotactic biopsy).

The heralding symptomatology in pineal lesions included headache, upper gaze palsy and signs of raised intracranial pressure; patients with suprasellar localization had a mild hypothalamic dysfunction syndrome, which led to neuroradiological examination. Cerebrospinal fluid (CSF) markers were sought including chorionic gonadotropin, α-fetoprotein and embryonal carcinoma antigen and were negative in all patients.

The stereotactic biopsy was performed by a right precoronal approach with the Riechert, Leksell or Cosman-Roberts-Wells frames [4]. After 1987, the procedure was assisted by computerized tridimensional reconstruction of the lesion and surrounding structures [11].

The histological features considered to confirm the diagnosis of germinoma included the presence of large polygonal cells (so-called syncytiotrophoblastic cells) and small round cells (so-called lymphoid-like cells) according to the World Health Organization (WHO) brain tumour classification [32]. The number of tissue samples within the target lesion was between 2 and 4 (mean 3). The potential proliferative activity was detected by (^{3}H)thymidine in vitro binding in specimens from 14 patients. The samples of tissues used in determining the labelling index (LI) were incubated in 2 ml of complete medium with (^{3}H)thymidine at 37 °C for 1 h in a shaking water bath. After incubation, the biopsy specimens were fixed in Bouin's solution for 1 h, embedded in paraffin and sectioned at 4 μm for an autoradiographic procedure. Deparaffined slides were obtained by the use of a stripping-film technique and exposed at 4 °C for 10 days. The slides were developed in Kodak D 19b (Eastman Kodak, Rochester, N.Y.) for 5 min at 18° C, fixed and stained with haematoxylin and eosin at 4° C. Lastly, the slides were examined by optical microscopy, and 1,000–10,000 cells were scored to count the total number of labelled cells, which represent the pool in the S phase of the cell cycle incorporating (^{3}H)thymidine in DNA. The LI was calculated as the ratio between labelled cells and total cells [8, 9, 14].

After the histological diagnosis, all but one patient underwent whole brain radiotherapy was first-choice treatment (54–79 Gy). The only patient who escaped this procedure was a 3-year-old child who was treated by systemic chemotherapy (etoposide, bleomycin, cisplatinum) to avoid the adverse effect of radiotherapy on the developing brain [1, 20].

Results

Morbidity from stereotactic biopsy resulted in massive intratumoral bleeding in one case, which lead to permanent upper gaze palsy. Operative mortality was zero.

The clinical examination and CT and MRI performed 3 months after treatment showed complete recovery from symptomatology and disappearance of the lesion in all patients (Fig. 1).

The long-term follow-up ranged from 3 to 13 years (mean 8) and was carefully recorded yearly for all patients by serial CT and MRI.

Three patients suffered fatal recurrence of the disease with CSF dissemination at 3, 4 and 2 years after the stereotactic biopsy and radiotherapy, respectively. The outcome in these patients was rapidly fatal in spite of intrathecal and systemic chemotherapy (intrathecal etoposide, systemic bleomycin and cisplatinum). Neoplastic cells detected within the CSF in these patients had an undifferentiated malignant appearance and could not be related to specific tumour lines. In one case, MRI performed at the time of progressive neurological worsening showed enlargement of the cervical spinal cord, suggesting parenchymal involvement by neoplastic dissemination (Fig. 2).

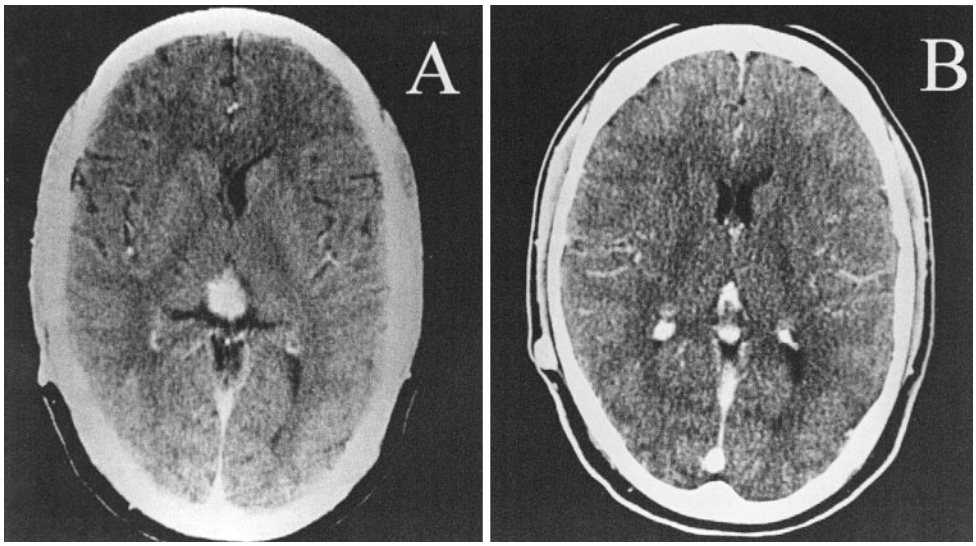

Fig. 1 A, B Contrast-enhanced CT of a 22-year-old male patient. The patient complained of upper gaze palsy and headache. Stereotactic biopsy of the pineal lesion revealed germinoma. **A** CT at the time of diagnosis. **B** CT 3 months after whole brain radiotherapy. This patient developed fatal CSF and neuraxis dissemination 4 years later

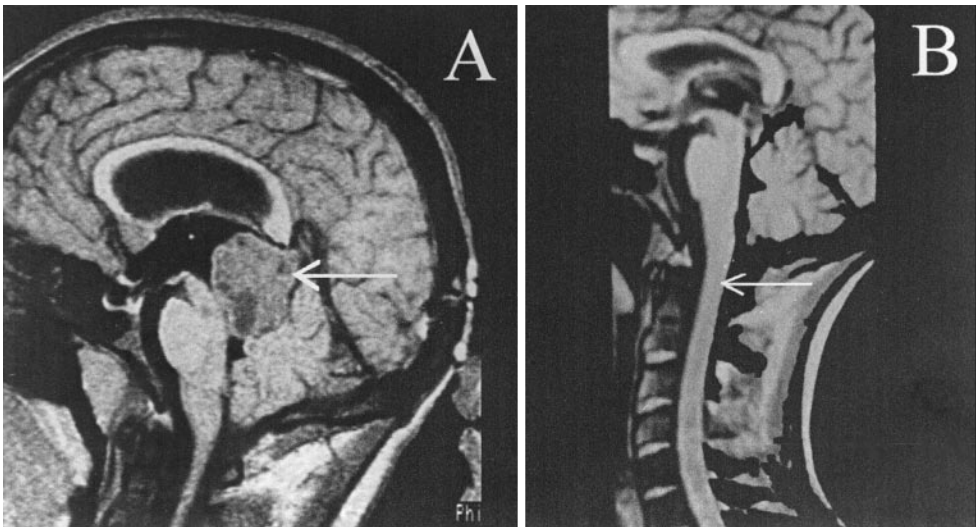

Fig. 2 A, B T1-weighted sagittal MRI of a 20-year-old male patient. The patient complained of raised intracranial pressure syndrome. Stereotactic biopsy of the pineal lesion revealed germinoma. **A** MRI at the time of diagnosis. **B** MRI 2 years later at the time of neuraxis dissemination. The *arrows* point to the pineal lesion in **A** and to the enlargement of cervical cord and medulla in **B**. Note the complete disappearance of the pineal germinoma at the time of fatal neoplastic CNS dissemination

The heralding symptoms of this syndrome were back and neck pain followed by motor impairment and finally by dramatic brain stem and cranial nerve involvement. Patients died within 3–4 weeks of the onset of the first symptoms. Two of these patients had a single pineal lesion and the third patient had an "ectopic" hemispheric single localization of germinoma. All of these patients had had previous successful whole brain radiotherapy.

The LI values in the whole series ranged between 0.1 and 5% (median 2.5). In patients who developed fatal CSF dissemination, the LI ranged from 1.9 to 2.1% without any significant prognostic indication compared with long survivors. It has to be pointed out that only syncytiotrophoblastic cells were labelled by (^{3}H)thymidine, while none of the small lymphoid-like cells showed thymidine labelling.

Discussion and conclusions

Stereotactic biopsy can be considered a reliable method to confirm histologically the presumptive neuroradiological diagnosis of germinoma [22]. The surgical removal of pineal lesions may be avoided if the CT or MRI appearance and the age of onset of the disease suggest the diagnosis of germinoma. Risks and benefits of stereotactic biopsy versus open surgery in patients harbouring lesions highly likely to be germinoma have not been matched in this study, but the morbidity in our series is considerably lower than the morbidity and mortality for open surgical procedures for pineal lesions [23, 24]. On the other hand, the surgical debulking of germinoma seems to be meaningless in tumours that have been proven to disappear after radiotherapy. In spite of these considerations, a tissue diagnosis is mandatory to plan the treatment and record the evolution and history of germinoma patients.

Nevertheless, the fatal recurrences of germinoma in our series were without predicting clinical, neuroradiological or biological features. The histological findings and the LI values were not particularly predictive in these patients. The CSF biological markers were negative for malignant disembryogenic tumours at the time of stereotactic biopsy [16]. The time lapse between stereotactic biopsy and recurrence ranged from 2 to 4 years. CSF dissemination occurred after a complete clinical and neuroradiological remission of the disease. This means that possible misunderstanding of the nature of the lesion owing to failure of stereotactic biopsy may be ruled out in our series. In other, words, a misleading biopsy of a germinoma-like cell population in a tumour also harbouring malignant disembryogenic cells would be followed by an early malignant evolution in spite of radiotherapy [31].

The data obtained by LI assessment confirm the hypothesis suggested by Sano [25] that the germinoma can-

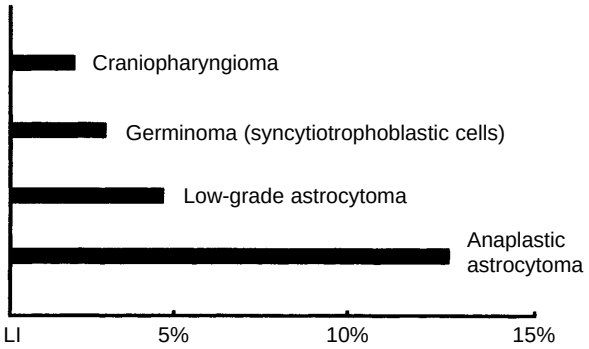

Fig. 3 Labelling index (*LI*) average values detected by (^{3}H)thymidine in vitro binding in specimens from stereotactic biopsies in germinoma patients compared with other intracranial lesions investigated by the same methodology and by the same authors [8]. Note that the germinoma LI valie is close to the slow-growing brain tumours' LI values. Only syncytiotrophoblastic cells were labelled by (^{3}H)thymidine in germinoma, while LI was zero in lymphoid-like cell populations

not be considered a true malignant tumour in spite of its radiosensitivity and in spite of its undifferentiated histological features. The occurrence of fatal outcome owing to dramatic recurrence and of highly malignant behaviour is not predicted by LI measured at the time of diagnosis.

In conclusion: stereotactic biopsy may be proposed as the first-choice method to confirm the neuroradiological diagnosis of germinoma, as recently suggested by an exhaustive cooperative study [24]. With regard to cell kinetics studies, germinoma lies in the area of slow-growing brain tumours (Fig. 3). Moreover, cell kinetics investigations suggest the non-neoplastic origin of lymphoid-like cell populations. They may explain the long persistence of these cells after radiotherapy, as reported in autopsy cases observed soon after irradiation [2]. The high radiosensitivity of germinoma does not correlate with the potential proliferative activity, as usually happens in radiosensitive brain tumours [7, 17]. The reasons for this behaviour (as well as the role of lymphoid-like cells) are still unknown. Radiotherapy does not prevent the development of fatal recurrence (15% in our series), which may occur years after the original treatment. The incidence of recurrence does not differ from reported series of patients who underwent craniospinal radiotherapy [29]. Brain radiotherapy, in our opinion, still remains the treatment of choice in histologically proven germinoma. Our homogeneous series may represent a historical one to compare with patients submitted to different treatment modalities such as chemotherapy [1], radiosurgery [7] and further developing procedures.

In spite of the expected favourable prognosis in patients affected by intracranial germinoma, this tumour still resembles Russian roulette for the patients and remains an enigma for neuropathologists and radiotherapists.

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