

Neuroblastoma. A study of the clinicopathological features influencing prognosis based on the analysis of 54 cases

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Summary. The retrospective analysis of 54 cases of neuroblastoma taken from the files of the Department of Pathology, University of Santiago Hospital, Spain, and the Ludwig-Aschoff Institute of Pathology, University of Freiburg, Germany confirmed the validity and significance of various clinical and histopathological features when trying to establish the prognosis and the proper therapeutic approach in a given case of neuroblastoma. When the age of the patients was compared to survival it was shown that all but three of the patients older than 2 years of age had died from tumor within ten months. In contrast, there was a 37.5% five-year survival rate among patients who were 24 months of age or younger at the time of diagnosis and treatment. The primary tumor was located in the adrenal gland in 27 cases (50%), in 9 cases (17%) the tumor was retroperitoneal but extra-adrenal, and in the remaining 18 patients (33%) the tumor arose from the paravertebral sympathetic ganglia. Adrenal primaries behaved in an extremely aggressive manner as all but three patients with tumors at this location were dead within 18 months. Retroperitoneal extra-adrenal neuroblastomas followed an almost equally poor outcome with only one five-year survivor (11%). In contrast, 49% of the patients with paravertebral neuroblastoma had survived five years and a further 33% were alive with shorter follow-up. According to histological criteria, there were 6 grade I tumors, 15 grade II and 33 grade III tumors in our series. All grade I tumors were clinical stage I at diagnosis and all are alive 2 to 3 1/2 years later. Grade II tumors were clinical stage 2, 3 or 4 and showed a 46% five-year survival. With the exception of three patients with paravertebral tumors, all patients with grade III neuroblastoma were clinical stage 3 or 4 when initially

seen and all were dead from tumor within ten months, with a five-year survival of 9%. It is concluded that the age at diagnosis, location of the primary tumor and histological differentiation, all of which are interrelated, are the most reliable clinicopathological features affecting prognosis and therapy in neuroblastoma.

Key words: Neuroblastoma, Histopathology, Prognostic factors

Introduction

Neuroblastoma, the second most common non-systemic malignant tumor of infants and young children, is an embryonal neoplasm of the sympathetic ganglia and adrenal medulla that originates from neural crest derivatives (Dehner, 1986; Triche, 1986; Ramón y Cajal Junquera, 1990). Neuroblastomas make up about 15% of all childhood cancers, only being exceeded in this age group by lymphoreticular malignancies and tumors of the central nervous system (Dehner, 1975; Kissane, 1975). These tumors are highly aggressive, invading locally and metastasizing widely predominantly to liver, bones and lymph nodes (Sandstedt et al., 1983; Shimada et al., 1984; Ramón y Cajal Junquera, 1990). Two-year survival rates range from 12% to 37% with most pediatric series reporting 30% (Makinen, 1972; Marsden and Steward, 1976; Carlsen et al., 1986).

Several cases have been reported of neuroblastoma maturing or differentiating towards benign ganglioneuroma (Dyke and Mulkey, 1967; Wilkerson et al., 1967; Makinen, 1972) thus leading to speculation as to whether the degree of histological maturity might be related to prognosis in neuroblastoma. The importance of the histological appearance was reported as long ago as 1914 by Whal (Whal, 1914) who stated that «... a high degree of histologic differentiation is in direct

relationship with a good prognosis, while with a lower degree of histologic differentiation the outlook is poor». In 1949, Stout, in grouping the malignant tumors of the sympathetic ganglion into partly differentiated ganglioneuromas and sympathicoblastomas, observed that metastases arose from all the tumors composed exclusively of undifferentiated sympathicoblasts and from 18% of the tumors which showed a mixture of cells varying from undifferentiated sympathicoblasts to fully differentiated ganglion cells (Stout, 1949). The results of Makinen (1972), Hughes et al. (1974), Sandstedt et al. (1983), Gansler et al. (1986) and Tsuda et al. (1987) have also contributed evidence that supports the theory that prognosis in neuroblastoma is closely related to the degree of histological differentiation. Besides being influenced by histopathological features, prognosis of patients with neuroblastoma has been linked to age at diagnosis (Jereb et al., 1984; Stephenson et al., 1986).

More recently, the introduction of flow cytometry (Gansler et al., 1986) and molecular pathology techniques (Brodeur and Fong, 1990) to the study of neuroblastoma has defined new prognostic factors. The activation of various oncogenes (Brodeur et al., 1986), *bcl-2* proto-oncogene being one of the most frequent (Reed et al., 1991; Castle et al., 1993), and the number of copies of the *N-myc* oncogene found in tumor cells (Seeger et al., 1985) have both been associated with a poor prognosis. Immunohistochemical analysis has also contributed to define the biological behaviour of children with neuroblastoma, with some reports relating the outcome of the disease with the expression by tumor cells of certain antibodies (Shimada et al., 1985; Zeltzer et al., 1986).

The present study was undertaken to determine to what extent certain histological and clinical features affect the biological behaviour and consequently the therapeutic approach in a given case of neuroblastoma.

Materials and methods

The surgical pathology and autopsy files of the Department of Pathology, University of Santiago Hospital, Spain, and Ludwig-Aschoff Institute of Pathology, University of Freiburg, Germany were reviewed in order to find all cases have been diagnosed as neuroblastoma in a 23-year period (1971-1993). After excluding patients in whom sufficient histological material or follow-up information were not available, 54 histologically confirmed cases were included in this study.

Harris' haematoxylin and eosin (H&E) and periodic acid Schiff (PAS) stained sections were reviewed in every case without prior knowledge of the age of the patient, location of the tumor and final outcome of the disease. An average of four blocks per case was studied. In cases in which there was disagreement or the diagnosis was doubtful, and in order to confirm the previous diagnosis, immunohistochemical staining was performed using the avidin-biotin-peroxidase complex

(ABC) (Hsu et al., 1981) technique and commercially available antibodies to neuron-specific enolase (NSE) (polyclonal, dilution 1:20, Dakopatts, Glostrup, Denmark) and neurofilaments (monoclonal, dilution 1:3000, Becton-Dickinson, Mountain View, C., USA) as both have been reported to be good neuroblastoma markers (Tsokos et al., 1984; Mukai et al., 1986; Osborn et al., 1986; Triche, 1986; Oppedal et al., 1987).

According to their degree of histological differentiation the tumors were classified into three groups as proposed by Hughes et al. (1974): Grade I. Tumors showing a mixed pattern of undifferentiated cells and scattered ganglion cells; Grade II, tumors showing a mixed pattern of undifferentiated cells and some cells showing evidence of partial differentiation towards ganglion cells as indicated by any of the following: a) vesicular nuclei, b) presence of nucleoli, c) increased cytoplasmic-nuclear ratio, and d) formation of cytoplasmic processes; and, Grade III, tumors consisting exclusively of undifferentiated cells without any evidence of maturation. Presence of rosette formation was not considered to be evidence of maturation as it was almost invariably associated with poorly differentiated tumors.

In addition to the histological features mentioned above, the clinical records were reviewed and the following information was obtained: age of the patient at diagnosis, location of the primary tumor, clinical stage of the disease as proposed by Evans et al. (1971), and follow-up, that is, whether the patient was alive at the time of the study, with or without recurrent or metastatic disease, or, if dead, the length of survival from the date of histological diagnosis to death from tumor.

Results

Age, sex and race

Age of the patients ranged from 4 to 72 months with an average of 28 months at the time of diagnosis. There was a male:female ratio of 1.6:1. All patients were Caucasians.

Anatomical location

The primary neoplasm was located in the adrenal gland in twenty-seven cases (50%) with a predilection for the left side (18 tumors were located in the left adrenal). The paravertebral sympathetic ganglia were the site of origin of the tumor in eighteen patients (33%), and in the remaining nine cases the tumor was located in the retroperitoneum, although the adrenal glands did not appear to be involved at the time of surgery.

Histopathological features

According to the histological criteria mentioned above, six tumors were included in grade I, consisting of

Prognostic factors in neuroblastoma

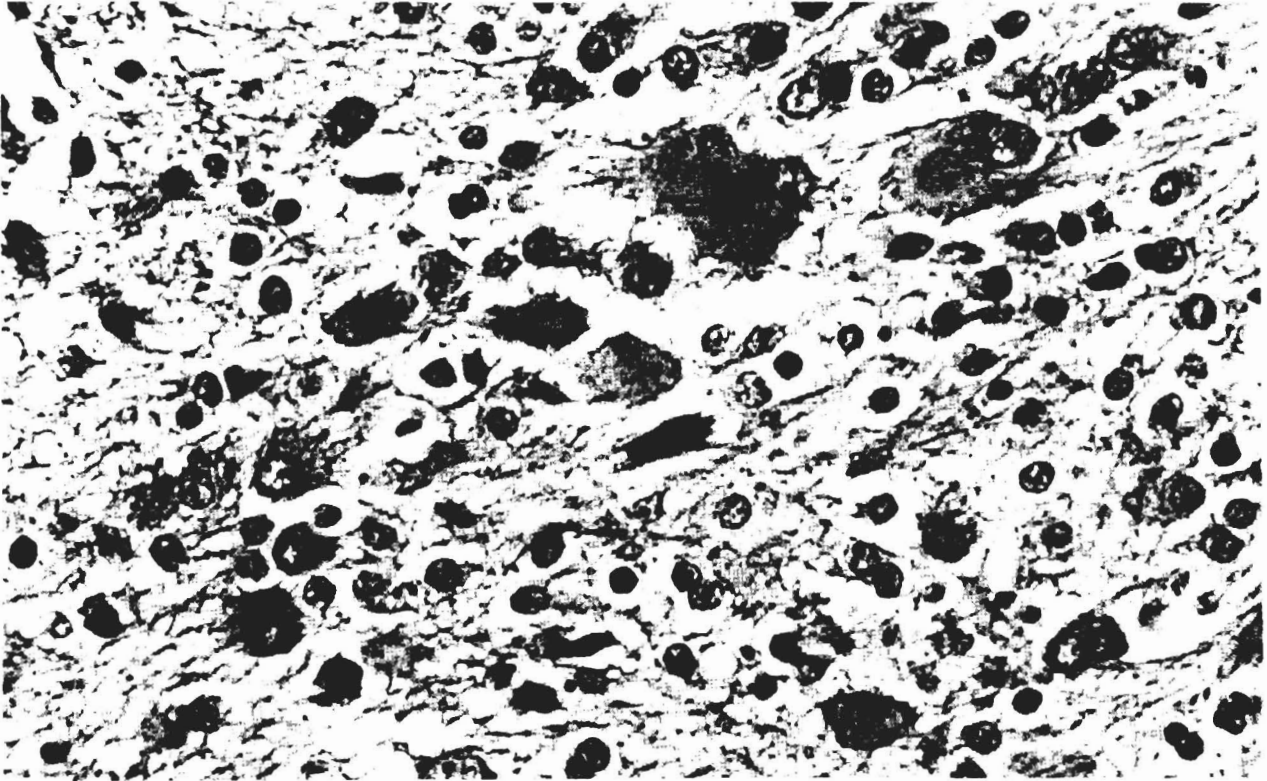


Fig. 1. Neuroblastoma grade I. The tumor is formed by an admixture of undifferentiated neuroblasts and mature ganglion cells. H&E. x 225

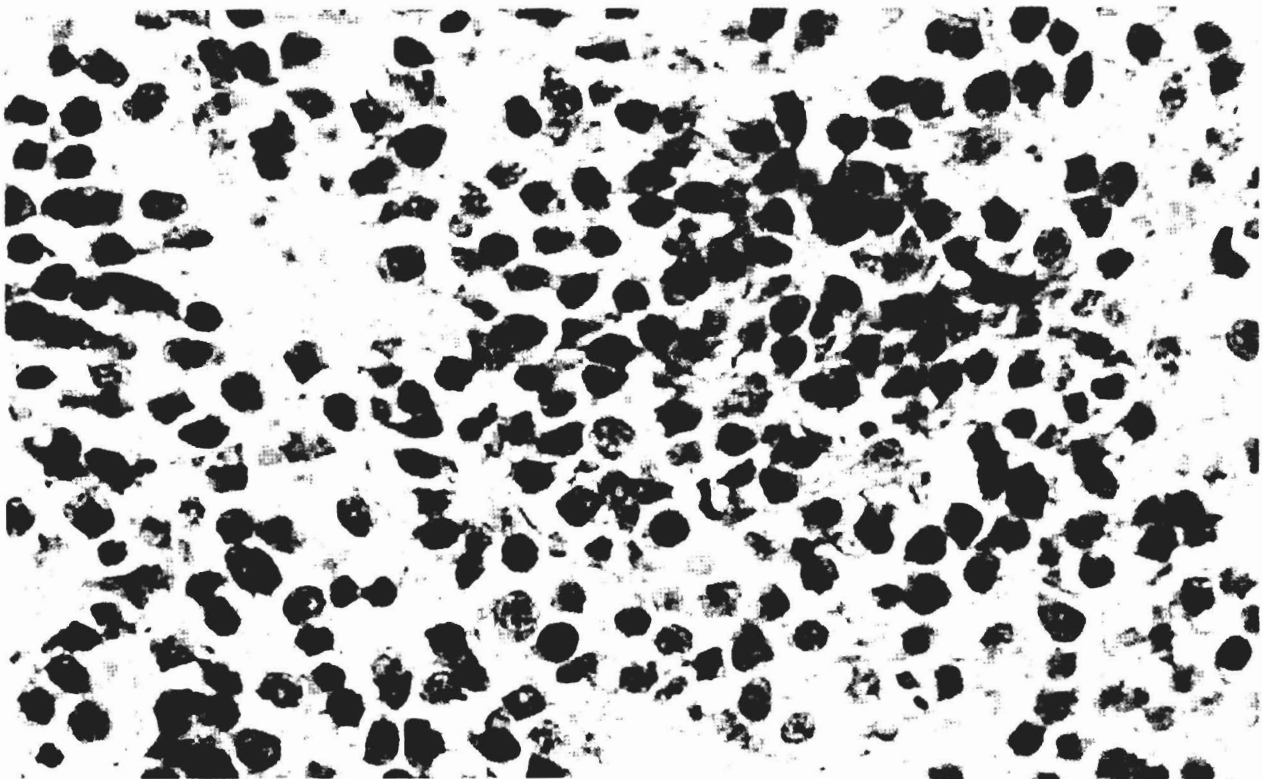


Fig. 2. Neuroblastoma grade II. Some of the tumor cells show evidence of maturation as indicated by vesicular nuclei and increased cytoplasmic-nuclear ratio. H&E x 225

a mixed proliferation of undifferentiated cells and mature ganglion cells (Fig. 1). Fifteen tumors were grade II, showing a mixed pattern of undifferentiated cells and some cells showing evidence of partial differentiation towards more mature ganglion elements (Fig. 2). This evidence of maturation was evaluated by the presence of large vesicular nuclei, or presence of an identifiable nucleolus, or an increased cytoplasmic-nuclear ratio, or identification of cytoplasmic processes, or a combination thereof. Finally, in thirty-three cases the tumors were classified as being grade III neuroblastomas and consisted of a proliferation of very immature neuroblasts without any evidence of differentiation (Fig. 3) and with very notorious rosette formation (Fig. 4).

Follow-up and prognostic factors

When the age of the patients was compared to survival (Fig. 5) it could be shown that all but three of the patients older than two years of age had died from tumor within ten months. In contrast, there was a 37.5% five-year survival rate among patients who were 24 months of age or younger at the time of diagnosis.

Adrenal primaries behaved in an extremely aggressive manner, as all but three patients with tumors at this location were dead within 18 months (Fig. 6). Retroperitoneal extra-adrenal neuroblastomas seemed to

follow an almost equally poor outcome with only one five-year survivor (11%). In contrast, 49% of the patients with neuroblastomas arising from the paravertebral sympathetic ganglia had survived five years and a further 33% were alive with a shorter follow-up (Fig. 6).

When compared to prognosis, it could be shown that the only six patients with grade I neuroblastoma were alive and with no signs of recurrent or metastatic disease from 2 to 3 1/2 years after diagnosis, respectively (Fig. 7). From the group of patients whose tumors were included in grade II, only three with adrenal primaries were dead from tumor within one year of diagnosis, and the overall five-year survival rate for this group was 46% with one of the surviving patients representing a well-documented case of spontaneous regression. Thirty-three patients were diagnosed as having grade III neuroblastoma and, with the exception of three cases with tumors arising from the paravertebral sympathetic ganglia, and another three cases in which follow-up was short, all patients were dead from tumor within ten months from diagnosis with an overall five-year survival rate of 9% (Fig. 7).

When the extent of the disease was clinically established, it was found that all cases of grade I neuroblastoma were clinical stage 1, grade II tumors were stages 2, 3 or 4 at diagnosis, and all but three grade III neuroblastomas were stages 3 or 4 when initially seen.

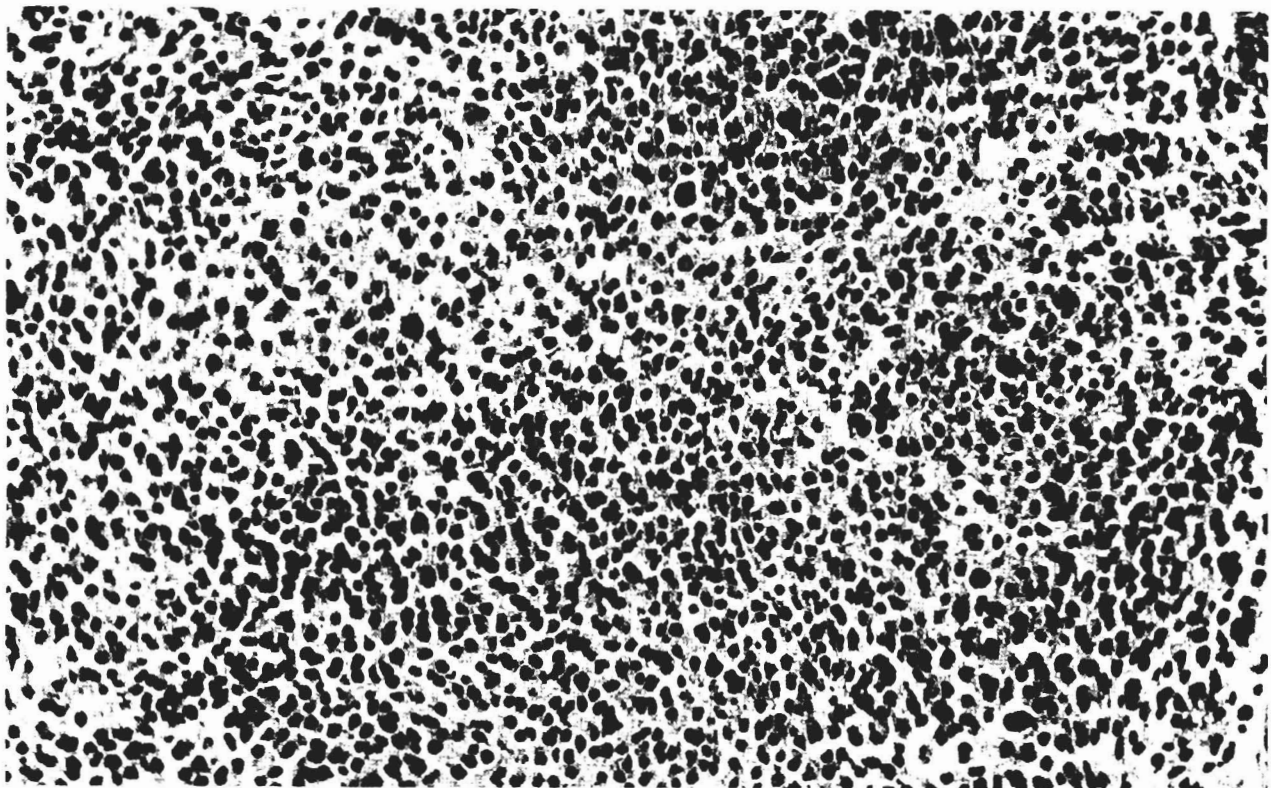


Fig. 3. Neuroblastoma grade III. The tumor is formed by undifferentiated neuroblasts with no evidence of maturation. H&E x 165

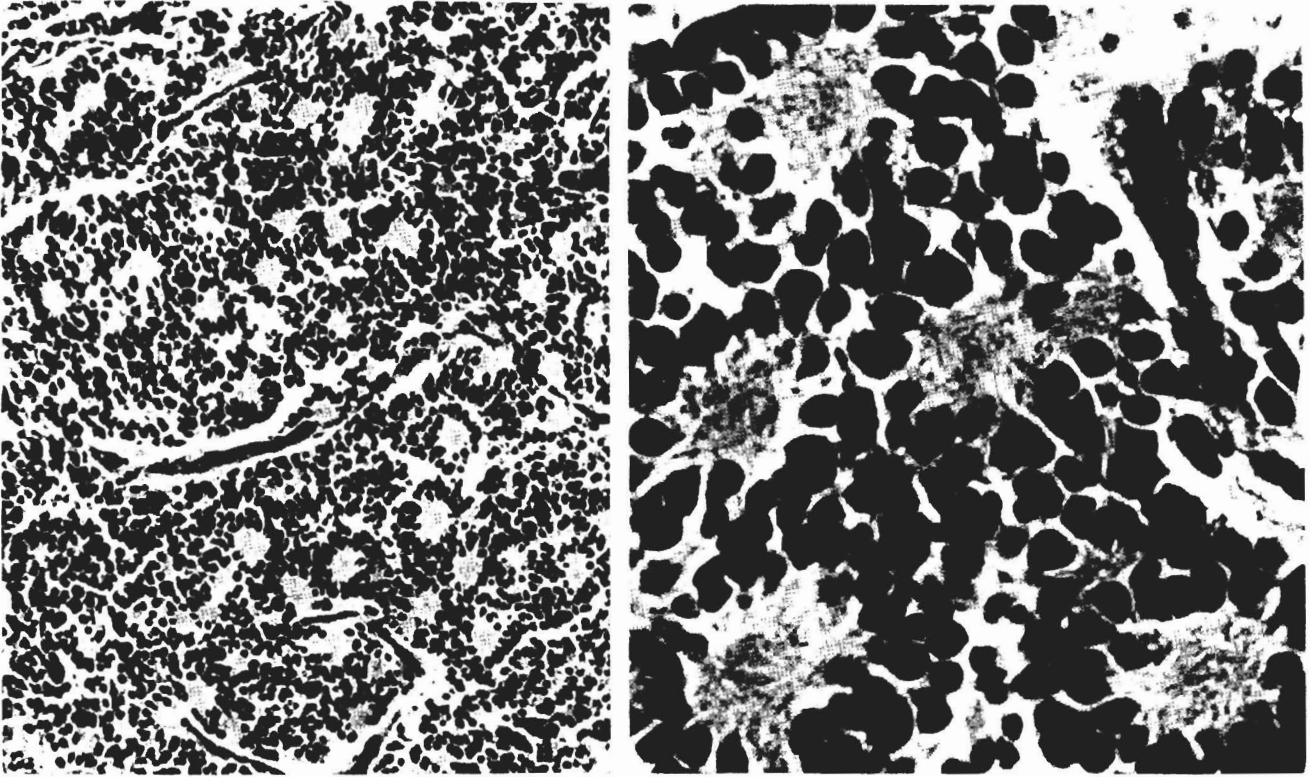


Fig. 4. a. Neuroblastoma grade III. Rosette formation in a retroperitoneal tumor. H&E. x 165. b. Higher magnification of typical rosettes formed by undifferentiated neuroblasts. H&E. x 420

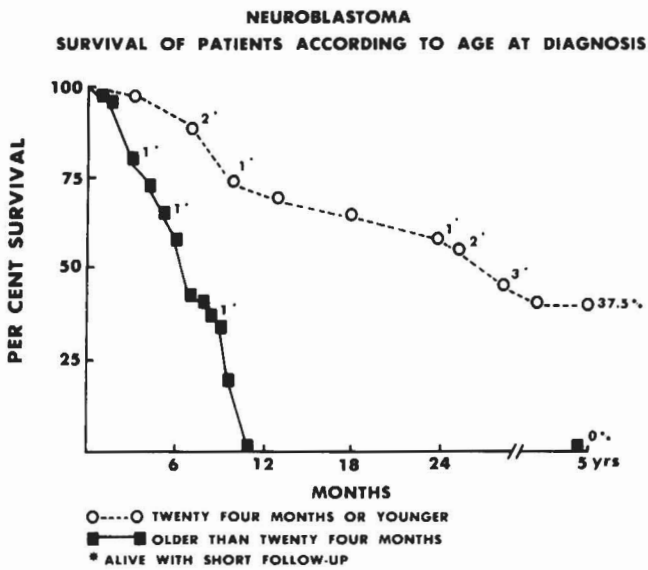


Fig. 5. Neuroblastoma. Survival of patients according to age at diagnosis.

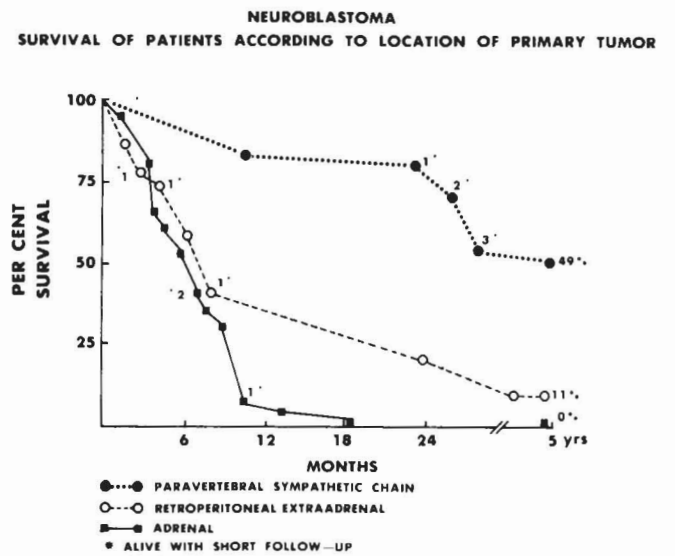


Fig. 6. Neuroblastoma. Survival of patients according to location of primary tumor.

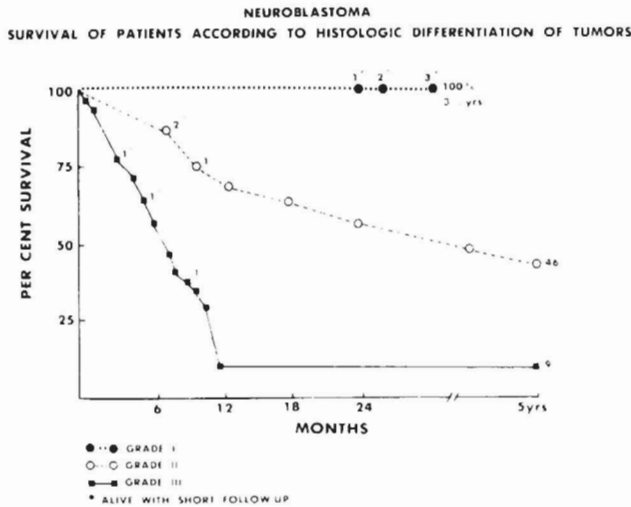


Fig. 7. Neuroblastoma. Survival of patients according to degree of histological differentiation.

With the exception of six patients, all grade III tumors were retroperitoneal primaries.

Discussion

There have been a number of previously reported studies on the significance of maturation in determining survival in patients with neuroblastoma (Beckwith and Martin, 1968; Hughes et al., 1974; Shimada et al., 1984, 1985; Gansler et al., 1986; Tsuda et al., 1987), all of which showed, quite conclusively, that there is a definite relationship between survival and the degree of histological differentiation within the tumor. However, these reports did not emphasize either the important role that various clinical features (e.g. age at diagnosis, location of primary tumor) play in prognosis, or the correlation that exists between certain clinical and histopathological parameters (e.g. location of primary and histological differentiation, clinical stage and histological differentiation). More recently, age at diagnosis has been related to clinical behaviour in children with neuroblastoma (Jereb et al., 1984; Stephenson et al., 1986).

There were six grade I tumors (12%), fifteen grade II (27%) and thirty-three grade III (61%) tumors in our series. These figures of histological distribution correlate well with those given by other authors (Makinen, 1972; Harms and Wilke, 1979; Shimada et al., 1985; Carlsen et al., 1986). All six patients with grade I neuroblastoma underwent surgical excision of their tumors with no postoperative therapy and are alive without evidence of disease from 2 to 3 1/2 years after diagnosis. Patients with grade II tumors are likely to follow a different clinical course depending mainly upon the location of the primary tumor. Six of the fifteen patients diagnosed as having grade II neuroblastoma are dead from tumor and all of these had a retroperitoneal tumor primary. A

further group of three patients is alive with short follow-up thus showing a 46% five-year survival rate for grade II neuroblastoma. With the exception of three patients with paravertebral tumors, all grade III neuroblastomas behaved in an extremely aggressive manner. Twenty-seven patients were dead from tumor within ten months from diagnosis and the remaining three patients are alive with intraabdominal disease but with a shorter follow-up. Length of follow-up is of the outmost importance in neuroblastoma if rigorous biological behaviour is to be assessed, since late recurrences and metastases frequently occur (Dannecker et al., 1983). When histological grading was contrasted with clinical staging, as proposed by Evans et al. (1971), there was an obvious relationship between degree of histological differentiation and extent of the disease. All grade I tumors were clinical stage 1, grade II tumors were clinical stage 2, 3 or 4, and, with the exception of three cases, all grade III tumors were clinical stage 3 or 4 when initially seen, including a paravertebral primary.

The degree of lymphocytic infiltration of the stroma was shown by some investigators to bear a significant positive correlation with length of survival in patients with neuroblastoma (Lander and Aherne, 1972). This could not be proven either in our series or in Hughes' cases (Hughes et al., 1974). Furthermore, Beckwith and Martin (1968) have pointed out that the distinction between lymphocytes and primitive neuroblasts may be extremely difficult especially in the less differentiated tumors.

It has also been stated that prognosis in neuroblastoma is more favourable in the younger age groups (Sandstedt et al., 1983; Jereb et al., 1984; Shimada et al., 1984; Stephenson et al., 1986). We were able to confirm this statement with our results, as there was a 37.5% five-year survival rate among patients who were two years of age or younger at the time of diagnosis in our series. In contrast, all patients older than two years of age at diagnosis have died from tumor within ten months, with the exception of three patients with short term follow-up information.

Neuroblastomas are made up of cells which are morphologically quite similar to embryonic precursors of peripheral ganglion cells of the nervous system (Dehner, 1986; Triche, 1986). These neuroblasts are normally present at the site of the sympathetic ganglia during embryonic and foetal life and may be present in the adrenal until puberty. Presumably either the malignant cells in a neuroblastoma have not come under the influence of inducing agents which would normally stimulate differentiation into ganglion cells, or the inductive process has been defective due to: a) a diminished quantity of these agents; b) a reduced sensitivity to inductive substances by neuroblasts; c) an improper timing of contact with organizing materials, or some similar abnormality. As a result, the cells have retained their ability to grow autonomously and invade adjacent tissues (Wilkerson et al., 1967). However, in a given case, neuroblasts could, at a later stage and due to

yet undetermined host factors, show increased sensitivity to inductive substances and differentiate into ganglion cells thus leading to spontaneous regression of the tumor. This was the case in one of our patients who had a grade II neuroblastoma with widespread skin metastases when first diagnosed. The tumor matured to ganglioneuroma and subsequently disappeared, and the patient is currently alive 14 years after diagnosis.

The phenomenon of spontaneous regression of neuroblastoma, which is said to happen in 5% of the cases (Dehner, 1975; Kissane, 1975), may take place in two different ways: maturation to a more differentiated tumor (benign transformation), or necrobiosis with subsequent fibrosis and focal calcification (regressive transformation). Although maturation of neuroblastoma to ganglioneuroblastoma or ganglioneuroma has often been discussed, only a few histologically verified cases have been reported (Wilkerson et al., 1967; Sandstedt et al., 1983; Carlsen et al., 1993). Regressive transformation in neuroblastoma, which may lead to surprisingly long survival and spontaneous cures even in cases with widespread metastases (stage IVs) (Lampert, 1974), is, however, almost exclusively confined to congenital neuroblastoma and to tumors diagnosed during the first year of life. The development of host defence mechanisms by an increased immunological response (appearance of killer lymphocytes?), which is indicated by an increase of lymphoblasts and lymphocytes in the tumor tissues and in the blood and bone marrow, appears to play a decisive role in this type of spontaneous regression of this malignant tumor (Bill and Morgan, 1970; Evans and Hummeler, 1973).

In addition to the above mentioned clinicopathological features, we have found that the location of the primary tumor is, probably, the single most reliable indicator of prognosis in children with neuroblastoma and, furthermore, bears a very close relationship to histological differentiation. There were 36 retroperitoneal tumors in our study, 27 of them having a well documented origin in the adrenal gland. Thirty of these 36 patients, including 24 of the 27 adrenal primaries, are dead from tumor after an average survival of 12 months. In contrast, there was a 49% five-year survival rate among patients with paravertebral tumors, a further 33% of patients are alive with short follow-up, and, among the survivors, there are three patients with grade III neuroblastomas arising from the paravertebral sympathetic ganglia. These results correlate with those of other authors (Hughes et al., 1974; Sandstedt et al., 1983; Carlsen et al., 1986) in whose series some of the patients with a grade III extraperitoneal tumors were also among the survivors. Although some authors do not emphasize the difference in distribution of histological grades among various sites (Marsden and Steward, 1976; Triche, 1986), we have found that all grade I tumors were extraabdominal while 81% of grade III tumors were initially retroperitoneal in location.

The explanation for this difference in clinical behaviour has been attributed to various patterns of

embryogenesis. Evidence of an inherent difference between tumors undergoing benign transformation and the more common persistently malignant neuroblastomas was provided by the observation that, with a single questionable exception, all reported transformed tumors have been found in a paravertebral position or some other extra-adrenal location (Goldman et al., 1965). These extra-adrenal sites are the most common locations for ganglioneuroblastoma and ganglioneuroma. In contrast, neuroblastomas are more frequently found in the adrenal, and adrenal neurogenic tumors are usually malignant. The greater frequency of benignancy in paravertebral tumors, as well as the earlier embryological development of paravertebral ganglia, correlates well with its location being relatively near in space and time to the site of origin of their embryonic precursors and to the source of embryonic inducers which would be expected to control their development (Wilkerson et al., 1967). Similarly, the higher incidence of malignant tumors in the adrenal and the later development of neural components of the adrenal gland parallel the greater spatial and temporal distance of adrenal neuroblasts from the central nervous system. Therefore, as mentioned above, transformation of a neuroblastoma into a more benign lesion could result from an abnormally delayed response to inducing substances, with maturation occurring only after a period of abnormal growth.

Our results indicate that the degree of histological differentiation, the location of the primary tumor and the age of the patient at the time of diagnosis are of the greatest importance when trying to establish prognosis and a proper therapeutic approach in patients with neuroblastoma. Poorly differentiated tumors showing no evidence of maturation towards ganglion cells should be aggressively treated, as should be patients with retroperitoneal primaries and patients diagnosed after two years of age, if prognosis of children with neuroblastoma is to be improved.

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Prognostic factors in neuroblastoma

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