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Neuron-specific enolase and malignant lymphomas (23 cases)

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Summary. The immunoreactivity of polyclonal antiserum to neuron-specific enolase (NSE) has been investigated. Twenty-three cases of malignant lymphoma (ML) were studied and compared with previously published reports. In our study 11 out of 23 cases showed strong or weak NSE positivity; any type of ML could be positive or negative even among B or T cell ML. This study indicated that polyclonal NSE is not a specific marker; it might be an inconstant marker of ML with no apparent correlation between reactivity and morphology or phenotype.

Key words: Neuron-specific enolase – Polyclonal antibody – Malignant lymphomas – Immunohistochemistry

Introduction

Enolases are cytoplasmic enzymes composed of three immunologically distinct subunits α, β and γ. Neuron specific enolase (NSE), the γ-γ dimer (Marangos et al. 1977) was first extracted from cerebral tissue. Recent immunohistochemical investigations have revealed that NSE is present in both neuronal and neuroendocrine cells (APUD system) (Schmechel et al. 1978; Tsokos et al. 1984). NSE has been demonstrated in neuro-endocrine derived cell tumours (Tapia et al. 1981; Dhillon et al. 1982; Wick et al. 1983; Sasaki et al. 1985; Leong 1986; Rubinstein 1986). However, a systematic study of antisera to NSE demonstrated a more evident positivity in miscellaneous tumours (Wick et al. 1983; Vinores et al. 1984; Kilidireas et al. 1986; Leader et al. 1986; Rubinstein 1986; Vinores et al. 1986). Malignant lymphomas (ML) have not been specifically investigated (Wick et al. 1983; Tsokos et al. 1984; Vinores et al. 1984; Battifora and Silva 1986; Wick et al. 1987) and there are discrepancies in the results reported. We therefore studied 23 cases of ML to precise the validity of NSE and compared our findings with the few previously published cases.

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Material and methods

Twenty-three cases of ML consisted of 15 biopsy specimens, seven surgical and one necropsy sample. The localizations were lymph nodes and spleen (nine cases), digestive tract (eight cases), central nervous system (C.N.S.) (five cases), and orbit (one case). The fixation was Bouin's liquid except for the formalin-fixed necropsy case. Immunohistochemistry was performed on three micron thick paraffin embedded sections.

Monoclonal antibodies (MAB) to epithelial membrane antigen (EMA) and to leucocyte common antigen (LCA) (Dako Corporation, Denmark) were studied by the three step method (Ancelin et al. 1984). The binding of polyclonal antiserum to bovine neuron-specific enolase (NSE) (Dako Corporation, Denmark) was detected using a standard Avidin-Biotin-Complex technique (ABC) (Vectastain kit, Vector, California, USA) (Hsu et al. 1981); this antibody reacts predominantly with γ subunits of neuronal origin with a possible cross-reactivity with β subunits (Dako Corporation, Denmark). The dilutions used were 1:25, 1:25, 1:1000 respectively. Revelation of the reaction used 3-3'-diaminobenzidine tetrahydrochloride (DAB). For negative controls, sections of each tumour were stained by omitting the primary antibodies. Sections of stock tissues and tumours known to contain the antigens of interest were used as positive controls.

Results

Nineteen ML were classified according to the Kiel classification (Lennert and Mohri 1978) and the two cases of Hodgkin's disease belonged to the type III of the Lukes-Rye's classification. Amongst the 19 ML, eight cases were recognized as large

Table 1. ML types and immunoreactivity of NSE

ML Types	Cases No.	B or T	NSE	NSE + and + + cases number
1. CLL	1	ND	0	0/2
	2	B	0	
2. Centroblastic-centrocytic follicular	3	B	+	1/1
3. Centroblastic diffuse	4	B	+	
	5	ND	0	
	6	ND	+ +	2/6
	7	B	0	
	8	B	0	
	9	ND	0	
4. Large cells				
immunoblastic	10	ND	+	
	11	ND	0	1/2
irregular	12	ND	0	
	13	T	+	
	14	T	+ +	
	15	T	0	5/8
	16	T	+ +	
	17	T	0	
	18	ND	+	
	19	T	+	
5. Histiocytic sarcoma and histiocytosis X	20		+ +	
	21		0	1/2
6. Hodgkin type III (Sternberg-Reed cell)	22		+ +	
	23		0	1/2

NSE +, + +, 0: neuron-specific enolase (weakly, strongly positive, negative); CLL: chronic lymphoid leukemia; ML: malignant lymphoma; ND: not determined

irregular cell ML: six were of T cell subtype and two were not subtyped. Five other cases were identified as B cell ML. There was one case of true histiocytic sarcoma and one case of histiocytosis X.

A positive LCA reactivity with anti LCA-MAB was expressed in all cases of non Hodgkin's ML except for one immunoblastic ML. Only one positivity with EMA antibody was present in a diffuse centroblastic ML. The results (Tables 1 and 2) showed that the intensity of the reactivity of NSE could be strong, weak or negative and was not correlated to the morphology or the subtypes B or T of the ML (Fig. 1a, b): the density of the cytoplasmic immunoreactivity was uniform in all the tumour cells in a given tumour. The same findings were observed for Sternberg-Reed cells and histiocytic or Langerhans' cells (Fig. 1c, d). Among the 23 studied cases (Tables 1 and 2), five, six and 12 cases respectively showed strong, weak and negative immunoreactivity. The only constant result was the negativity of the central nervous system in ML (biopsy and post-mortem material).

Discussion

Several authors (Tapia et al. 1981; Dhillon et al. 1982; Vinores et al. 1984) have demonstrated that NSE immunoreactivity was elevated in neuronal and neuroendocrine tumours; in non neuroendocrine tumours it was negative. Schmechel et al. (1978) thus suggested that the presence of NSE would be very useful in determining the neuroendocrine nature of a tumour. Subsequently the presence of NSE was detected in melanomas (Dhillon et al. 1982; Hirano 1986), in neuroblastomas (Triche and Askin 1983), in breast, lung and ovary carcinomas (Wick et al. 1983; Vinores et al. 1984; Kilidireas et al. 1986; Leader et al. 1986; Rubinstein 1986), in rhabdomyosarcomas (Leader et al. 1986; Rubinstein 1986), in giant cell tumour of tendon sheaths (Vinores et al. 1984) and in chordomas (Vinores et al. 1984). However, Battifora et al. (1986) reported negative results in their series of melanomas, lymphomas and undifferentiated carcinomas and still considered NSE to be a valuable marker of neuroendocrine tumours.

Table 2. NSE reactivity, sites and types of ML

Sites	NSE	ML types	Cases No.
CNS	0	Centroblastic diffuse	7 8 9
	0	Large irregular cells	12 17
Lymph nodes/spleen	++	Hodgkin type III	22
	+	Centroblastic-centrocytic follicular	3
	+	Large irregular cells	18
	0	CLL	1 2
	0	Centroblastic diffuse	5
	0	Large cells: immunoblastic irregular	 11 15
	0	Hodgkin type III	23
Digestive tract	++	Centroblastic diffuse	6
	++	Large irregular cells	14 16
	+	Centroblastic diffuse	4
	+	Large cells immunoblastic irregular	 10 13 19
	0	Histiocytosis X	21
Orbit	+	Histiocytic sarcoma	20

NSE +, ++, 0: neuron-specific enolase (weakly, strongly positive, negative); ML: malignant lymphoma; CLL: chronic lymphoid leukemia; CNS: central nervous system

Few reports investigating ML have been published (Wick et al. 1983; Tsokos et al. 1984; Vinores et al. 1984; Battifora et al. 1986). In our study 11 out of 23 cases showed NSE positivity. In the literature all the previously published cases have also been studied on paraffin embedded material. Positive NSE reactivity has been observed in one case of large cell lymphoma (Wick et al. 1983) (Peroxidase-anti-peroxidase (PAP), non commercialized antiserum, unknown fixation). Negative reactivity was found in an imprecise type of ML (Vinores et al. 1984, PAP, Marangos' antiserum 1:500 to 1:2000 diluted, formalin fixation), in a diffuse large centroblastic cell ML (Dhillon et al. 1982, indirect method, non commercialized antiserum, formalin fixation), in six lymphomas (Tsokos et al. 1984) in three cases of lymphoblastic lymphomas, one case of undifferentiated non Burkitt's lymphoma, two cases of histiocytic lymphomas – PAP and ABC, Marango's and Polysciences antisera, formalin and Bouin's fixation), in 20 large cell ML (Battifora et al. 1986 – ABC, Dako antiserum, formalin fixation, trypsinization) and in 113 small cell ML (Wick et al. 1987, PAP, Dako antiserum 1:400, formalin fixation). In these five studies, antisera were prepared by the authors except for the two last where our antiserum was used. In a recent study (Battifora et al. 1986) trypsin digestion, which generally enhanced the reaction, gave negative results. The discrepancies between previous studied and ours cannot be explained by the different fixatives, as even with the Bouin's fixation, the positivity was inconstant. It is noteworthy that the antigenicity of the γ enolase in normal lymphocytes was markedly reduced in formalin-fixed, paraffin-embedded sections (Haimoto et al. 1985). Moreover this reactivity did not seem to follow a general rule, as any type of ML could be positive or negative for NSE; even among B and T cell ML, there was no clear predominance. Furthermore the five ML of CNS had no reactivity for NSE. This has to be correlated with the results

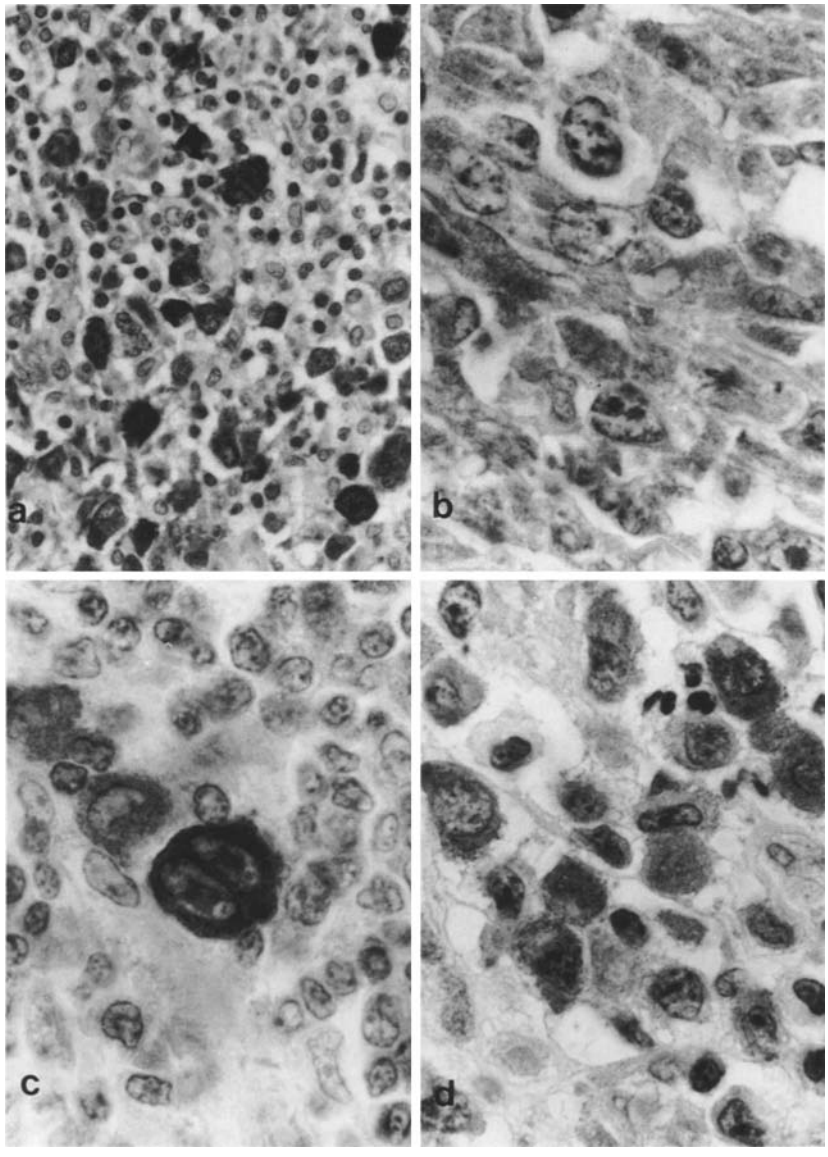

Fig. 1. Immunoperoxidase staining with anti-neuron-specific enolase polyclonal antibody. **a** Intense cytoplasmic positivity of large irregular cells in a T cell malignant lymphoma. × 384. **b** Weak cytoplasmic staining of malignant immunoblastic cells. × 960. **c** Strong cytoplasmic staining of a Sternberg-Reed's cell. × 960. **d** Marked cytoplasmic staining in an histiocytic sarcoma. × 960

obtained in vitro by Pählman et al. (1986) who observed that expression of NSE is a characteristic feature of B and T cells at a particular differentiation stage. Recently a MAB (H 14) has been prepared (Soler Federsppiel et al. 1987), against the γ subunit with no cross reactivity with the α or β subunits, and positive controls were obtained with paraffin embedded tissues. The future utilization of such an antibody will probably permit to explore the true value of γ enolase as a cellular marker.

In conclusion this study shows that the γ polyclonal antibody developed against NSE is not a specific marker as previously reported and may be an inconstant marker of ML, with no apparent correlation between reactivity, morphology and phenotype.

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