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Discrimination of Leukemias, Lymphomas, and Non-Neoplastic Controls by Retroviral Serum Markers

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A. Introduction

Diagnostic and prognostic markers are urgently needed for early diagnosis and therapy of malignant neoplasias. One solution of this problem is the search for etiologic factors in the hope that they might indicate the presence or degree of progression of the disease. One possible factor are the retroviruses which are known to cause certain malignancies in several animal species [1]. These malignancies include leukemias, lymphomas, and sarcomas. Footprints of retroviruses have also been repeatedly described in man, and retroviral isolates have been grown from human neoplastic and non-neoplastic cells. These viral isolates include not only the human T lymphotropic viruses (HTLV-I–III), but also viruses that are very similar to the primate retroviruses simian sarcoma virus (SiSV) and baboon endogenous virus (BaEV) [2, 3, 6].

We have shown over the past few years that antigens and antibodies that are related to structural proteins of these primate viruses are present in human sera [4] and possibly possess prognostic relevance. For instance, one can correlate the presence of antigens and immune complexes that are related to the envelope glycoprotein gp70 of SiSV with shorter survival and poorer response to therapy in acute leukemias and chronic myelogenous leukemias in blast crisis [5]. We have therefore examined whether such retroviral markers, possibly in combination, can be utilized for diagnostic purposes.

B. Materials and Methods

I. Patients

Sera from patients with leukemias, lymphomas, and from control subjects without neoplastic disease were treated with 5% trasylol and stored at −20 °C. If possible, sera obtained at diagnosis or prior to treatment were used.

II. Antigens and Antibodies

p30 core proteins and gp70 envelope glycoproteins were prepared from purified SiSV and BaEV as described [7, 8]. Polyvalent antisera were prepared by injecting the purified viral antigens into rabbits [7]. Monoclonal antibodies (MoAB) against BaEV gp70 were the gift of Dr. L. Thiry, Liège, Belgium.

III. ELISA

The examination of the sera for cross-reacting antigens, antibodies (IgG and IgM), and immune complexes (IgG and IgM) was done with the enzyme-linked immuno-

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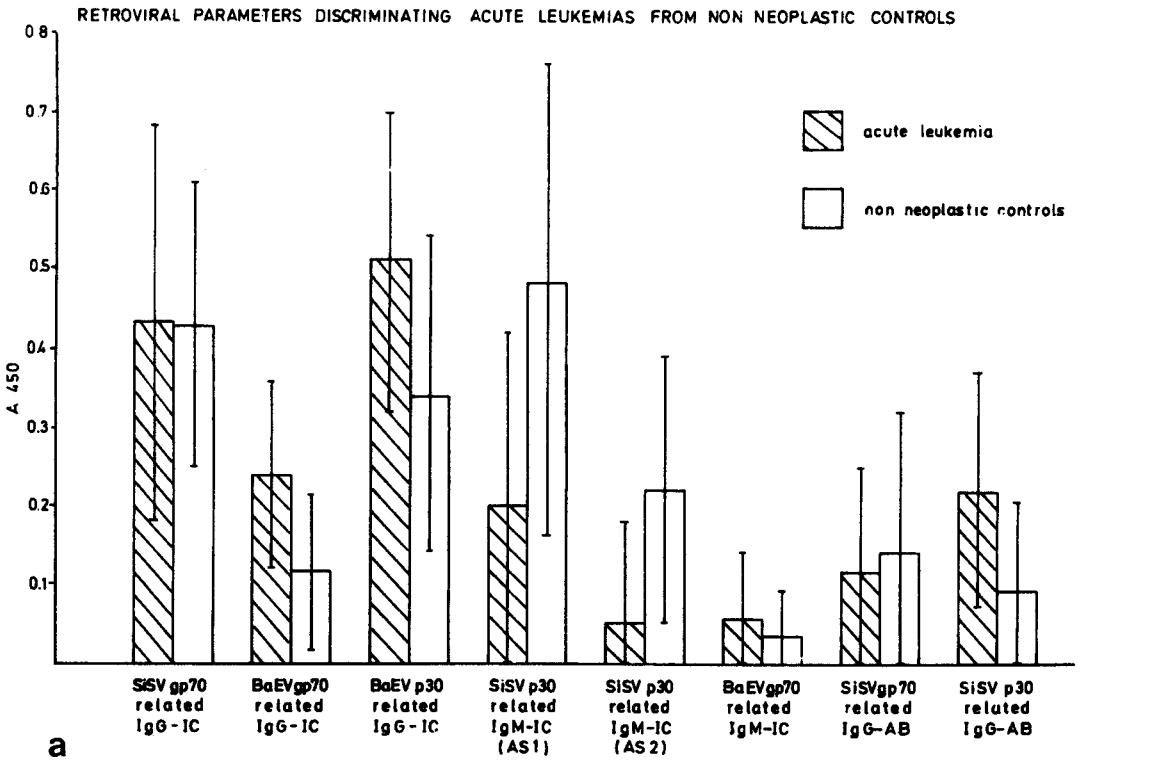

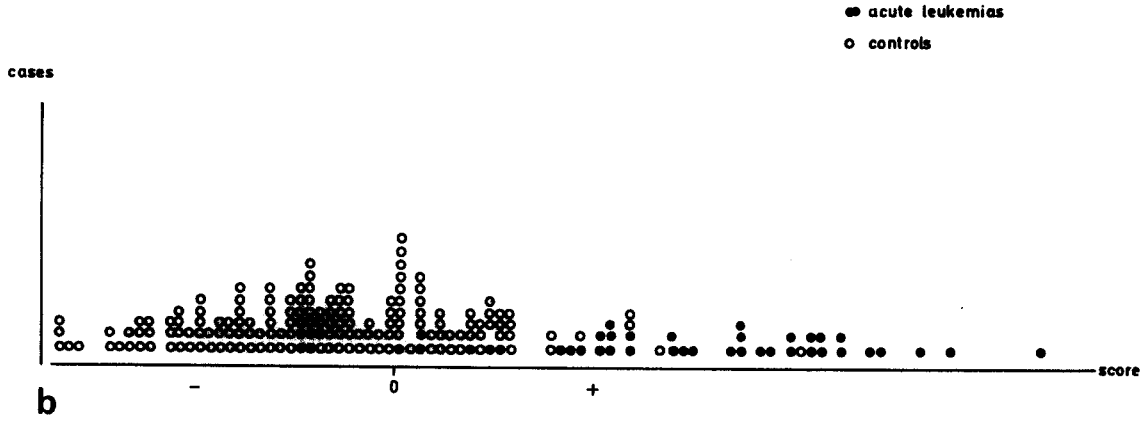

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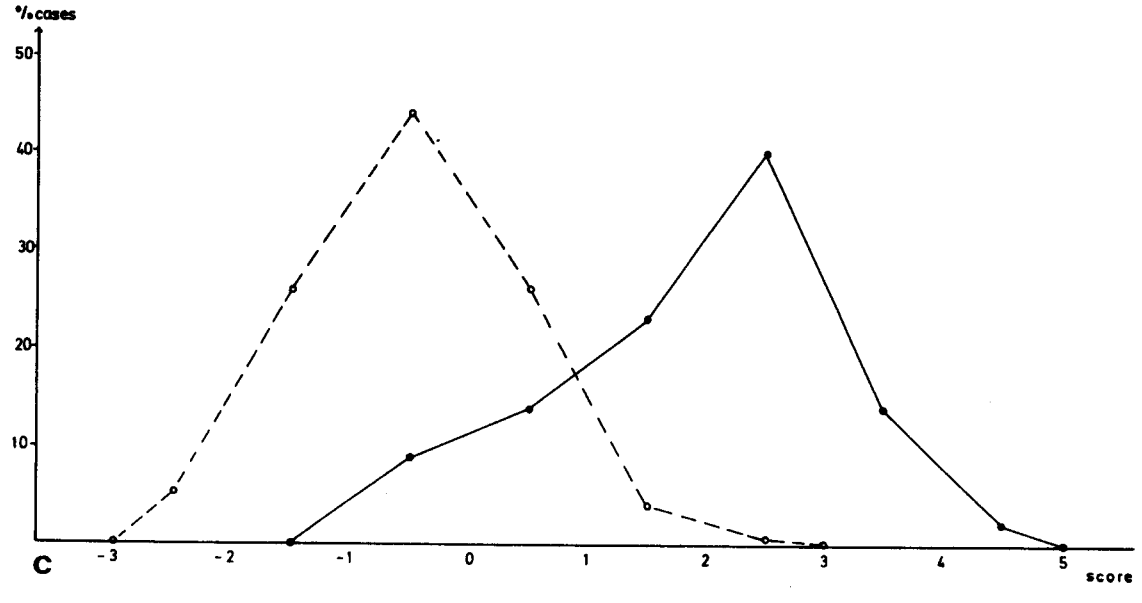

Table 1. Parameters for the discrimination of acute leukemias

a) Acute leukemias ($N=43$) vs controls ($N=154$)		
Parameters		
Immune complexes:	SiSV gp70 IgG	Sensitivity 82.4%
	BaEV gp70 IgG	Specificity 88.1%
	BaEV p30 IgG	
	SiSV p30 IgM (AS 1)	
	SiSV p30 IgM (AS 2)	
	BaEV gp70 IgM	
Antibodies	SiSV p30 IgG	
	SiSV gp70 IgG	
b) Acute leukemias ($N=43$) vs malignant lymphomas ($N=46$)		
Parameters		
Antigen	BaEV gp70 (MoAB)	Sensitivity 78.3%
Immune complexes	SiSV p30 IgG	Specificity 90.7%
	BaEV p30 IgG	
Antibodies	SiSV gp70 IgG	
	SiSV p30 IgG	
	BaEV gp70 IgG	
c) Acute leukemias ($N=43$) vs sarcomas ($N=17$)		
Parameters		
Antigen	BaEV gp70 (MoAB)	Sensitivity 100.0%
Antibodies	SiSV gp70 IgG	Specificity 97.7%
	SiSV p30 IgG	
	SiSV gp70 IgM	

sorbent assay (ELISA) technique [7, 8]; Kreeb et al., in preparation). For the detection of antigen-specific immune complexes, assays with immune and with preimmune sera were carried out in parallel. The extinction values obtained with the preimmune sera were subtracted from the values obtained with the immune sera (Kreeb et al., in preparation).

C. Results

We examined serum samples from 43 patients with acute leukemias, and from 46 patients with malignant lymphomas for antigens, antibodies (IgG and IgM), and immune complexes (IgG and IgM) that cross-react with the p30 core proteins and the gp70 envelope glycoproteins of SiSV and of BaEV and tried to distinguish the sera from each other and from three control groups (154 non-neoplastic controls, 10 benign lymphadenopathies, and 17 sarcomas). The enzyme-linked immunosorbent assay (ELISA) technique served as test system. The assay results were then subjected to stepwise discriminant analysis, and a score was determined. The results are shown in Tables 1 and 2.

Fig. 1a–c. Stepwise discriminant analysis of acute leukemias versus non-neoplastic controls. **a** means and standard deviations of the test results of each test parameter with acute leukemias and with controls; **b** scores calculated from the results with the test parameters for each individual leukemic and control sera; **c** distribution curves of leukemias and controls

I. Acute Leukemias

Acute leukemias can be distinguished from non-neoplastic controls with a sensitivity of 79.1% and a specificity of 90.9% (20.9% false negatives and 9.1% false positives) by eight parameters (Table 1a). The parameters are: IgG immune complexes related to SiSV gp70, BaEV gp70, and BaEV p30;

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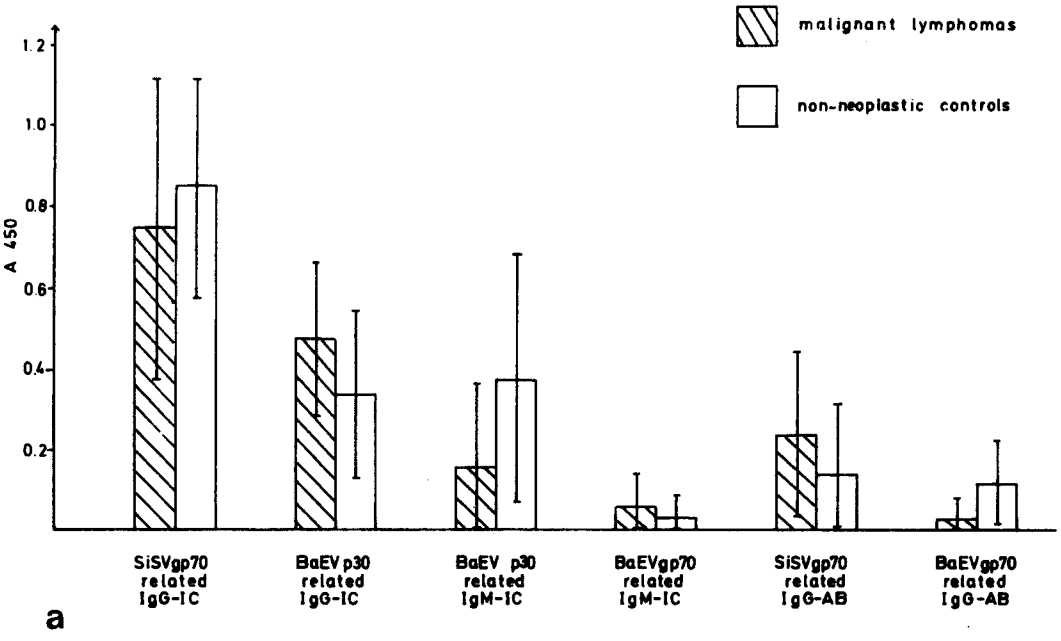

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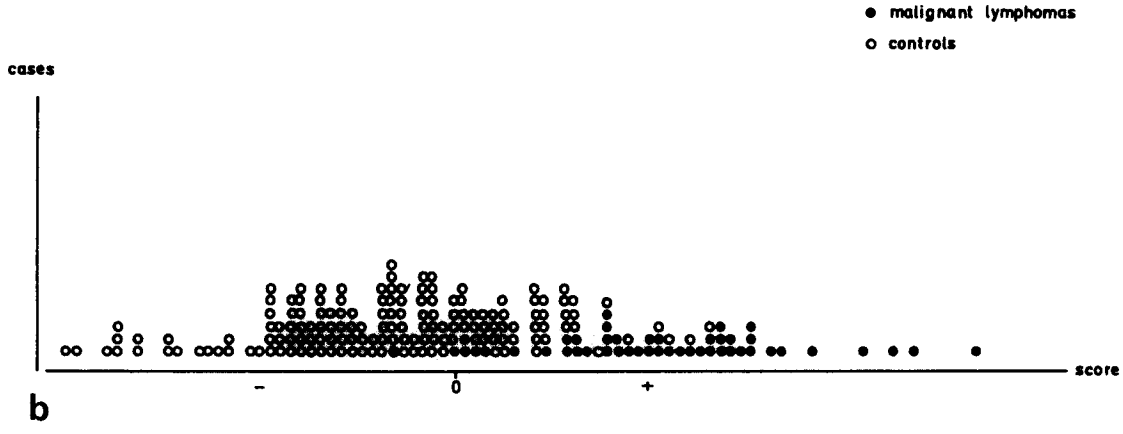

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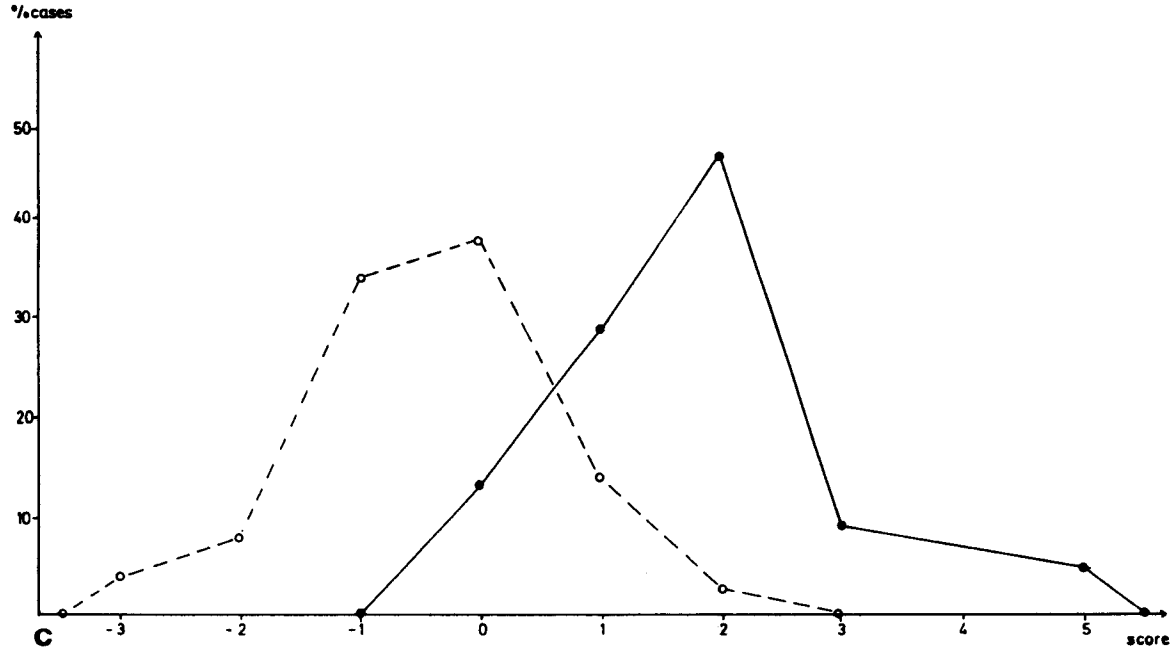
DISCRIMINATION OF MALIGNANT LYMPHOMAS● FROM NON-NEOPLASTIC CONTROLS○



c

Table 2. Parameters for the discrimination of malignant lymphomas

a) Malignant lymphomas ($N=46$) vs controls ($N=154$)		
Parameters		
Immune complexes	SiSV gp70 IgG	Sensitivity 84.4%
	BaEV p30 IgG	Specificity 83.3%
	BAEV p30 IgM	
	BaEV gp70 IgM	
Antibodies	SiSV gp70 IgG	
	BaEV gp70 IgG	
b) Malignant lymphomas ($N=46$) vs benign lymphomas ($N=10$)		
Parameters		
Antigen	BaEV gp70 (MoAB)	Sensitivity 97.8%
Immune complexes	BaEV p30 IgG	Specificity 70.0%
	BaEV p30 IgM	
Antibodies	SiSV gp70 IgM	
c) Malignant lymphomas ($N=46$) vs sarcomas ($N=17$)		
Parameters		
Antibodies	SiSV gp70 IgM	Sensitivity 100.0%
	BaEV gp70 IgG	Specificity 94.1%
Antigen	BaEV gp70 (MoAB)	
	SiSV gp70 (rabbit AB)	
Antibodies	SiSV gp70 IgG	

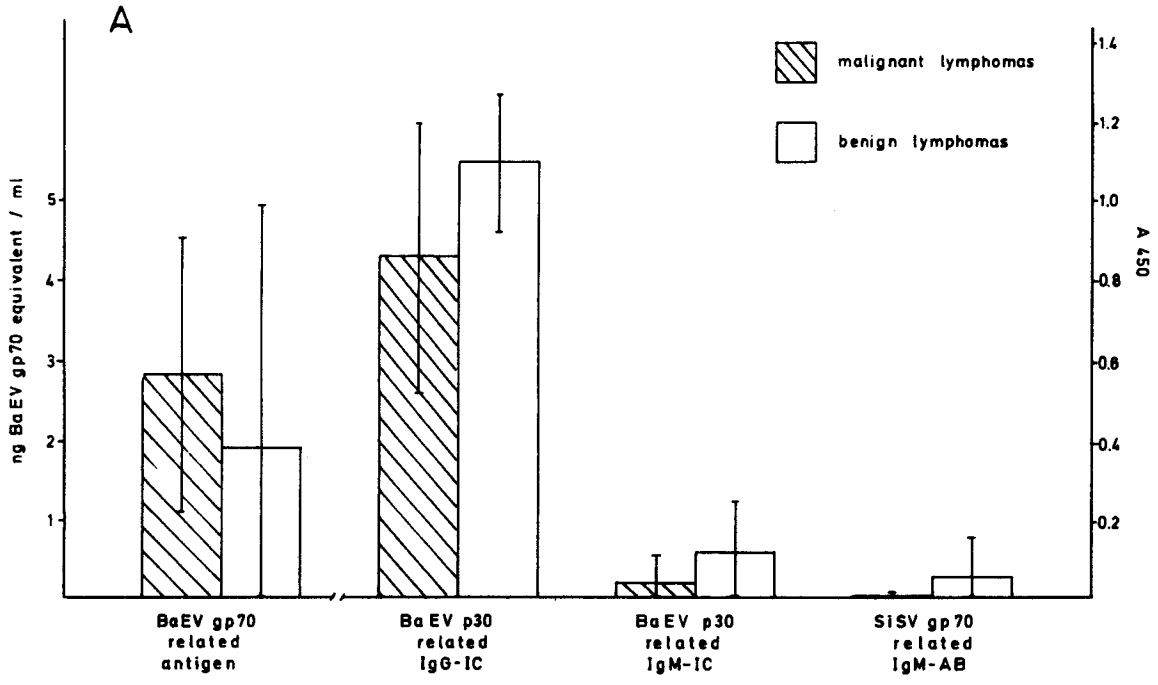
IgM immune complexes related to SiSV p30 and BaEV gp70; and IgG antibodies cross-reacting with SiSV gp70 and SiSV p30. The means and standard deviations of the test results of each parameter with the acute leukemia group and with the control group are shown in Fig. 1 a. The scores that are assigned to each leukemic and control serum were plotted and are shown in Fig. 1 b. The good separation of the two groups is evident. In Fig. 1 c, the numbers of patients per score unit are plotted, showing the relatively small overlap of the two groups. Similarly, acute leukemias were distinguished from malignant lymphomas with a sensitivity of 78.3% and a specificity of 90.7% by six parameters, and from sarcomas with a sensitivity of 100% and a specificity of 97.7% by the combination of four parameters (Table 1 b and c).

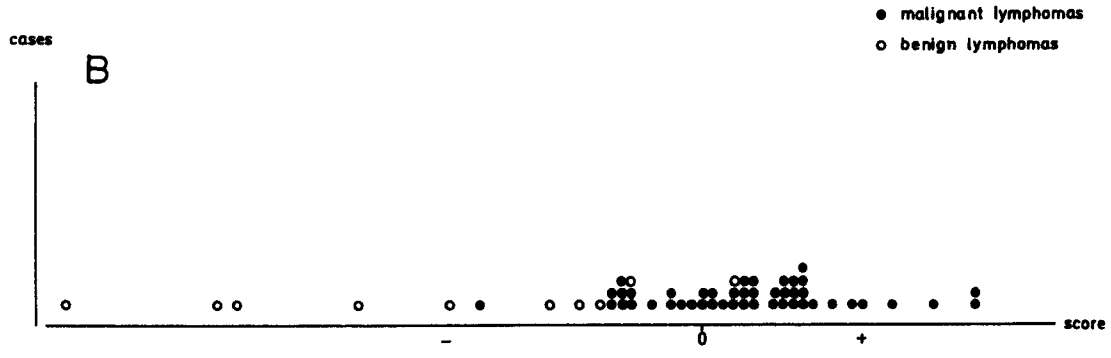
Fig. 2a–c. Stepwise discriminant analysis of malignant lymphomas versus non-neoplastic controls. **a** means and standard deviations of the test results of each test parameter with malignant lymphomas and controls; **b** scores calculated from the results with the test parameters for individual lymphoma and control sera; **c** distribution curves of lymphomas and controls

II. Malignant Lymphomas

Malignant lymphomas are distinguished from non-neoplastic controls with a sensitivity of 84.4% and a specificity of 83.3% (15.6% false negatives and 16.7% false positives) by six parameters (Table 2 a). The parameters are: IgG immune complexes related to SiSV gp70; IgG and IgM immune complexes related to BaEV p30; IgM immune complexes related to BaEV gp70; and IgG antibodies cross-reacting with SiSV gp70 and with BaEV gp70. The means and standard deviations of the test results of each of the six parameters with the malignant lymphoma group and with the control group are shown in Fig. 2 a. Figure 2 also shows the scores of each serum as individual values (Fig. 2 b) and as curves (Fig. 2 c) as described. The distinction between malignant lymphomas and benign lymphadenopathies is depicted in Table 2 b and Fig. 3. Malignant lymphomas are distinguished from benign lymphadenopathies with a sensitivity of 97.8% and a specificity of 70% by four parameters (Table 2 b). The four parameters are: BaEV gp70-related antigen (as determined by monoclonal antibodies); IgG and IgM im-

RETROVIRAL PARAMETERS DISCRIMINATING MALIGNANT LYMPHOMAS FROM BENIGN LYMPHOMAS





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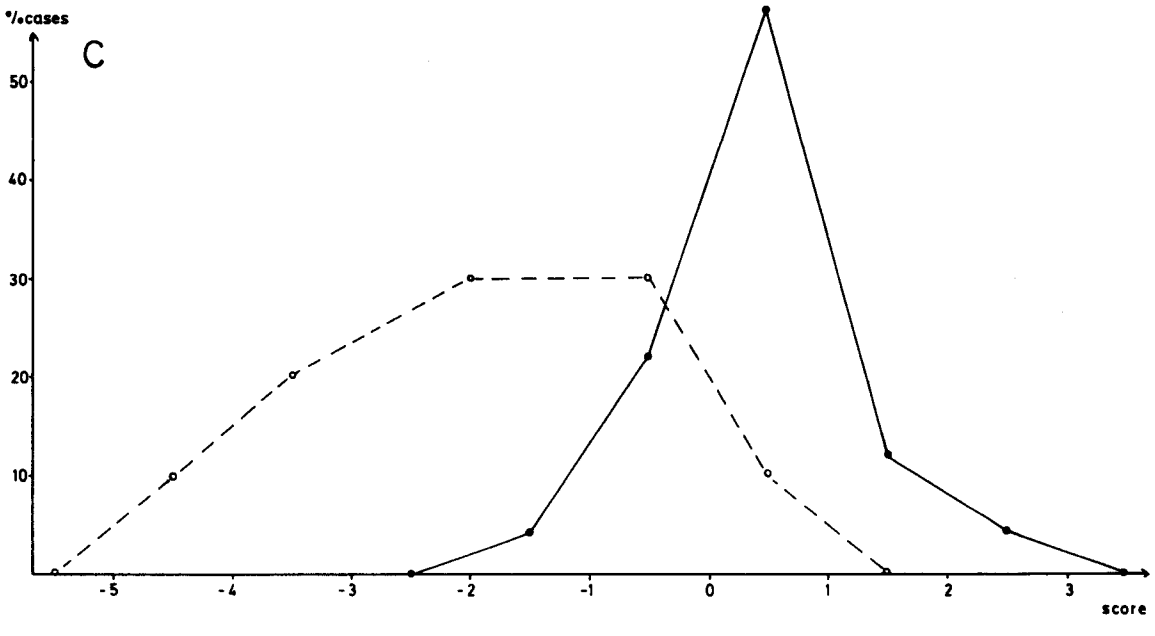

Fig. 3 A–C. Stepwise discriminant analysis of malignant lymphomas versus benign lymphadenopathies. **A** means and standard deviations of the test results of each test parameter with malignant and benign lymphomas; **B** scores calculated from the results with the test parameters for individual lymphoma sera; **C** distribution curves of malignant and benign lymphomas

mune complexes related to BaEV p30; and IgM antibodies cross-reacting with SiSV gp70. Means and standard deviations of the test results with the individual test parameters (Fig. 3a), calculated scores as individual values (Fig. 3b), and as curves (Fig. 3c) are shown as already explained. Sarcomas are distinguished from malignant lymphomas with a sensitivity of 100% and a specificity of 94.1% by five test parameters (Table 2c): BaEV gp70-related antigen as determined by monoclonal antibodies; SiSV gp70-related antigen (by rabbit antiserum); IgG antibodies cross-reacting with BaEV gp70 and SiSV gp70; and IgM antibodies cross-reacting with SiSV gp70.

D. Discussion

On the basis of animal experiments, studies have been performed for several years to elucidate the etiologic role of retroviruses in human cancers, especially leukemias and lymphomas [1]. The first human candidate virus HTLV-I has been isolated and described by Gallo and co-workers [2], which can be regarded as the cause of T cell leukemias in humans in endemic areas. The detection of SiSV- and BaEV-related viruses or structural components of these primate viruses in human tissues or sera has been described independently by several groups [2, 3]. Their origin and their relevance for human malignancies has not been elucidated. Their ubiquitous appearance in humans makes it reasonable to regard them as of endogenous origin. This is supported by the description of homologous gene sequences to MuLV and BaEV [9, 10]. As with other endogenous retroviral entities, their etiologic role in tumors is likely, but far from being proven.

In human leukemias, we have detected a significant coincidence between the presence of cross-reacting envelope proteins of SiSV/SSAV and a poor prognosis and resistance to therapy [5]. We now describe the possibility of distinguishing certain human malignancies of mesenchymal origin on the basis of the presence of a number of primate retroviral markers. These include viral related antigens and antibodies and especially virus-specific immune complexes. The discrimination between the different patient groups or between patient groups and the control group could only be achieved with a certain number of parameters, but discrimination was unexpectedly high in comparison with other parameters used as special tumor markers. It is interesting to note the dominant role of virus-specific immune complexes of IgG and IgM classes in every discriminating pattern. This further supports the possible role of immune complex formation in the course of malignant diseases.

These results are a promising step in the direction of better diagnosis and more detailed classification of certain mesenchymal tumors. A prospective study is necessary to establish these parameters as diagnostic and prognostic factors. This may also be improved by the use of monoclonal antibodies, which is indicated by the fact that BaEV gp70 as detected by a monoclonal antibody has been included twice in discrimination patterns. Last, but not least, the prognostic and diagnostic relevance of the retroviral structures described here can be used as an argument for their possible etiologic significance in mesenchymal neoplasias.

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