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RESEARCH ARTICLE

ROLE OF PAX-5 IMMUNOHISTOCHEMISTRY IN HAEMATOLYMPHOID MALIGNANCIES

[1,*]Shruti Bansal, [2]Sunita Singh, [3]Rishu Garg, [4]Yashika Bhatia, [5]Mansi Aggarwal, [6]Manpreet Tanwar and [7]Rajeev sen

[1]PG Resident, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak, Haryana
[2]MD Senior Professor, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak, Haryana
[3]MD Senior Resident, Department of Medicine, MAMC, New Delhi
[4]MD Senior Resident, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak, Haryana
[5]MD Senior Resident, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak, Haryana
[6]PG Resident, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak, Haryana
[7]MD Head of Department, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak,

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ABSTRACT

Objective: Histomorphology and immunohistochemistry are essential tools for evaluation and classification of haematolymphoid malignancies. The present study was conducted with the aim to study and categorize various haematolymphoid malignancies in lymph node and bone marrow biopsies using routine H & E staining and IHC markers and to study usefulness of Pax-5 in differentiating various lymphomas and leukemias. **Material and Methods**: The present study was conducted in Department of Pathology at PGIMS, Rohtak. Seventy cases of haematolymphoid malignancy including lymph node biopsy and trephine bone marrow biopsy were included in the study. Various histomorphological changes were examined on routine H&E. Cases with provisional diagnosis of lymphoma/leukemia were further submitted to immunohistochemicalstaining for a panel of lymphoma/leukemia markers including Pax-5 immunohistochemical antibodies. A descriptive study was carried out for all the variables included in the study. Fischers exact test was used to compare the categorical values. P-value <0.05 was accepted as statistically significant. **Results:** Immunohistochemical expression of Pax-5 was detected in all cases of Diffuse large B-cell lymphoma (n=9), Small lymphocytic lymphoma (n=7), Peripheral B Cell Lymphoma-NOS (n=6), follicular lymphoma (n=5), mantle cell lymphoma (n=4), marginal zone lymphoma (n=1) and B cell-ALL (n=8). In Hodgkin Lymphoma, nuclear Pax-5 immunoreactivity was seen in 71.4 % (5/7) of cases.However, Pax-5 was not detected in any case of NHL-T cell type including Anaplastic Large cell lymphoma (n=2) and T cell-ALL (n=1), AML (n=9) and plasma cell neoplasm (n=4).One of the two cases of ALCL was initially kept with differentials of ALCL or cHL but was subsequently reclassified as ALCL based on negative Pax-5 immunoexpression. The expression of Pax-5 in all the cases was statistically significant.(p<0.05). **Conclusion:** Pax-5 is an excellent B cell marker with its immunohistochemistry being a valuable tool in the diagnosis and subclassification of haematolymphoid malignancies.

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INTRODUCTION

Haematolymphoid malignancies constitute one of the important group of cancers in India, as elsewhere in the world. They are a group of heterogeneous malignant conditions with their incidence on upsurge in many regions and populations during the last few decades. The reasons for increasing incidence are multiple, ranging from the increasing elderly population, greater accuracy of diagnosis and unknown environmental factors. They comprise lymphomas, leukemias, myeloproliferative neoplasms, plasma cell dyscrasias, histiocytic tumors, and dendritic cell neoplasms. The diagnosis of haematolymphoid malignancies can be made from a number of different specimens like lymph nodes, bone marrow aspirates, trephine biopsy cores, peripheral blood as well as other fluids such as cerebrospinal fluid (CSF), ascitic fluid and pleural aspirates, depending on the presenting clinical features. Histomorphology and immunohistochemistry are essential tools for arriving at diagnosis of these malignancies (Rodig, 2015) Morphologic assessment taking into account the anatomic architectural alterations in the lymphoid compartment, presence of an abnormal population (polymorphic or monomorphic), determination of pattern (diffuse or nodular) and cell size and nuclear characteristics, sometimes alone is not sufficient for achieving diagnosis as there is overlap of morphologic features in certain undifferentiated and anaplastic haematolymphoid malignancies.

Corresponding author: **Shruti Bansal,**
PG Resient, Department of Pathology, Pt. B.D. Sharma PGIMS Rohtak, Haryana.

In such circumstances, using a panel of antibodies is an extraordinarily powerful tool for identifying the specific lineage and accurate subtype (Rao, 2010). Immunohistochemistry (IHC) is cost effective and can be performed quickly in routine practice. The initial panel of markers applied based on morphological differential diagnosis - leukocyte common antigen (LCA), CD20 and CD79a as B-cell markers, CD3 and CD5 as T-cell markers and other markers like CD15, CD30, CD23, bcl-2, CD10, cyclinD1, ALK-1 and CD138 based on cytoarchitectural pattern is usually sufficient to arrive at diagnosis. However, aberrant expression of a single T-cell associated antigen on Diffuse large B-cell lymphoma is occasionally seen. Also classical Hodgkin Lymphoma (cHL), a tumor of B-cell lineage that is CD45 negative, often lacksexpression of pan B-cell markers (CD20 and CD79a) and/or may paradoxically express T-cell antigens, thus, making it difficult to distinguish from Anaplastic large cell lymphoma (ALCL), a T-cell neoplasm. Also certain other B-cell neoplasms such as Precursor B-lymphoblastic leukemia/ lymphoblastic lymphomas are frequently negative for CD 20 and approximately 20% of patients of B-cell lymphoma following rituximab therapy experience relapse with CD20-negative tumor lacking expression of mature B-cell markers thus making diagnosis difficult. In such conditions, using Pax-5 immunoreactivity in diagnostic samples of haematolymphoid malignancies can help establish B-cell lineage (Desouki, 2010) We studied histomorphological changes and various immunohistochemical (IHC) markers including Pax-5 to categorize various haematolymphoid malignancies.

MATERIALS AND METHODS

Seventy cases of haematolymphoid malignancy including lymph node biopsy and trephine bone marrow biopsy were included in the study. A representative biopsy was received for every case. The specimens were grossly examined and fixed. The tissue was processed and paraffin embedding was done by routine histological technique. Specimen of bone marrow trephine biopsy was fixed in formalin, decalcified in 5% EDTA (ethylenediamminetetraacetate) and processed to paraffin-wax embedding. Histopathological diagnosis was established on routine H&E stain. Cases with provisional diagnosis of lymphoma/leukemia were further submitted to immunohistochemical staining for a panel of lymphoma/leukemia markers. These cases were subjected to Pax-5 immunohistochemical antibodies and usefulness of this marker was assessed in the diagnosis and further classification of lymphomas and leukemias. IHC was performed as follows: Paraffin sections (3-5 µm in thickness) mounted on slides with suitable tissue adhesivewere processed for - deparaffinization in xylene and rehydration through graded alcohols. Endogenous peroxidase enzyme was blocked by using 3% hydrogen peroxidase for 15-20 minutes. Antigen retrieval was done with microwave oven heating, for 30 minutes, with citrate. This was followed by incubation of sections with the monoclonal antibody (pre-diluted) (DAKO) overnight at 4°C. The sections were then rinsed with TBS solution followed by incubation with the secondary antibodies. The reaction was visualized using DAB (3,3′-Diaminobenzidine), and counterstaining ofnuclei was done using with hematoxylin. Positive and negative controlswere run with each batch of immunohistochemical stain. Positive control for Pax-5 were sections from tonsil and splenic tissue. Negative control was obtained by substitutionofthe primary antibody with an antibody of nonspecific positivity.

Interpretation of results: The appearance of nuclear immunoreactivity for Pax-5 was scored as positive or negative. Immunohistochemical result for Pax-5 protein was interpreted as positive, when the nucleus of neoplastic cells was positive.

Statistical Analysis: A descriptive study was carried out for all of the variables included in the study. Fischers exact test was used to compare the categorical values. p-value less than 0.05 was accepted as statistically significant.

RESULTS

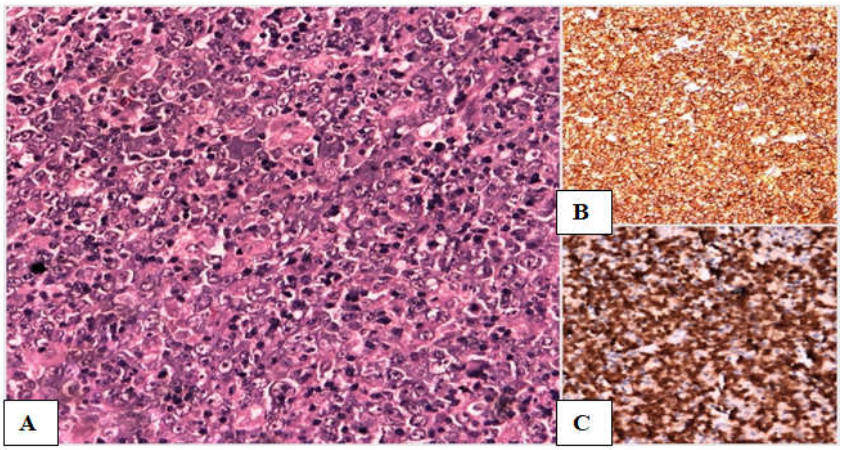
Age range in cases diagnosed with haematolymphoid malignancies was 1-80 years with the mean age of 49.9 years. Maximum numbers of cases (n=32) were seen between the age group of 30-60 years (45.7%). Males were predominantly affected constituting 71.5% of cases (n=50) and females about 28.5% (n=20) with a male:female ratio of 2.5:1.Out of total 70 cases, 42 cases were of lymph node biopsy accounting for 60% of cases and 28 were of bone marrow biopsy accounting for 40% of cases. Maximum number of cases included in the study were of Non Hodgkin Lymphoma- B cell type (n=32) constituting 45.8% cases followed by leukemias – AML (n=9), ALL (n=9) and Biphenotypic leukemia (n=1) together constituting 27.1% cases.NHL- T cell lymphomas (n=8) constituted 11.8% of the cases out of which 62.5% of cases were of peripheral T cell lymphomas (n=5), 25% of anaplastic large cell lymphomas (n=2) and 12.5% of lennerts lymphoma (n=1).Hodgkin lymphoma (n=7) and Plasma cell neoplasm (n=4) constituted 10% and 5.8% of cases respectively. Amongst Non Hodgkin Lymphoma- B Cell Type, DLBCL and SLL/CLL together constituted approximately 50% of the total cases and Peripheral B cell lymphoma - NOS constituted 18.7%, Follicular lymphoma 15.6%, Mantle cell lymphoma 12.5% and Marginal zone lymphoma 3.2% of the total cases respectively. All (100%) cases of DLBCL, SLL, Peripheral B Cell Lymphoma-NOS, follicular lymphoma, mantle cell lymphoma and marginal zone lymphoma showed positive immunoexpression for both CD20 and Pax-5 and hence the expression of Pax-5 was statistically significant ($p<0.05$) with 100% sensitivity (Figure. 1 and 2).CD10 positivity was seen in all 5(100%) cases of follicular lymphoma, 3 out of 6 (50%) cases of DLBCL, 1 out of 6 cases (16.6%) of SLL whereas no expression was seen in cases of mantle cell lymphoma and marginal zone lymphoma. CD5 and CD3 were positive in all (100%) cases of NHL-T cell type. Pax-5 and CD20 were, however, negative in all the cases and hence the absence of Pax-5 immunoexpression in T cell lymphomas was statistically significant. ($p<0.05$).Two cases of Anaplastic large T- cell lymphoma included in the study were positive for CD30 and CD5 but were ALK-1 negative. Immunostaining for CD20 and Pax-5 was negative in both cases. However, one of these two cases was initially kept with differentials of ALCL or cHL but was subsequently reclassified as ALCL based on negative Pax-5 immunoexpression. Out of the 8 cases of B-ALL, only 5 cases (62.5%) showed positive immunoexpression for CD20. However Pax-5 showed positive immunoreactivity in all 8 (100%) cases of B-ALL (Figure. 3). The expression of Pax-5 was, thus, found to be statistically significant ($p<0.05$) by Fischers Exact test with 100% sensitivity. A case of T-ALL included in the study showed negative immunoexpression for Pax-5. All 9 (100%) cases of AML showed positivity for MPO and CD117 immunomarkers but were negative for Pax-5 immunoexpression. A case of biphenotypic leukemia showed positivity of Pax-5 in 2-3% cells.

Table 1. Expression of Pax-5 in haematolymphoid malignancies

	Diagnosis	Total cases	No. Of positive cases	Percentage (%)
NHL- B cell type	DLBCL	9	9	100
	Small lymphocytic lymphoma	7	7	100
	Peripheral B cell lymphoma- NOS	6	6	100
	Follicular lymphoma	5	5	100
	Mantle cell lymphoma	4	4	100
	Marginal zone lymphoma	1	1	100
NHL-T cell type	Peripheral T cell lymphoma- NOS	5	0	0
	Lennerts lymphoma	1	0	0
	ALCL	2	0	0
Leukemias	B ALL	8	8	100
	T ALL	1	0	0
	AML	9	0	0
	Biphenotypic leukemia	1	1	100
Hodgkin lymphoma		7	5	71.4
Plasma cell neoplasm		4	0	0

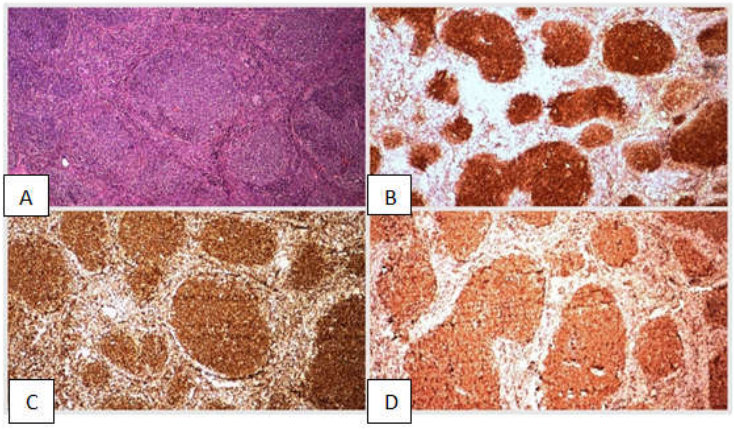
Table 2. Comparison of Pax-5 immunoexpression in haematolymphoid malignancies with other studies

Study	B cell NHL	T cell NHL	B cell ALL	AML	Hodgkin lymphoma	Plasma cell neoplasm
Desouki *et al*. (2010)	77/77 (100%)	0/22 (0%)	5/5 (100%)	0/4 (0%)	36/41 (88%)	-
Zhang *et al*. (2003)	66/70 (94.2%)	1/26 (3.8%)	10/14 (72.4%)	13/26 (50%)	-	0/6 (0%)
Krenacs *et al*. (1998)	83/102 (81%)	0/23 (0%)	-	-	7/18 (38.8%)	-
Fauceglia *et al*. (2007)	108/118 (91.5%)	0/7 (0%)	-	-	60/70 (85.7%)	-
Torlakovic *et al*. (2002)	96%	0%	-	-	97%	0%
Dong *et al*. (2008)	-	-	31/31 (100%)	-	80/86 (93%)	0/17 (0%)
Tiacci *et al*. (2004)	-	-	150/150 (100%)	15/160 (9.3%)	-	-
Present study	32/32 (100%)	0/8 (0%)	8/8 (100%)	0/9 (0%)	5/7 (71.4%)	0/4 (0%)



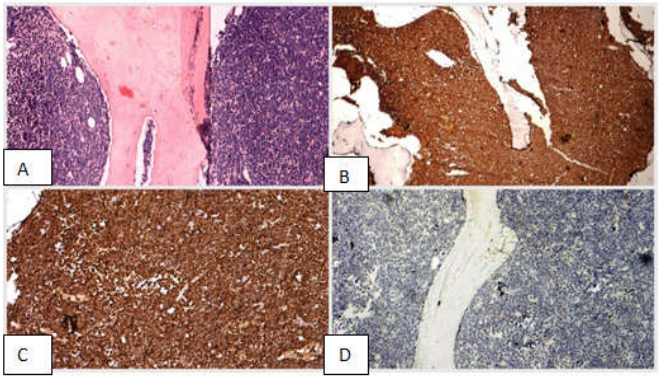
A. Photomicrograph showing effacement of nodal architecture by tumor cells (Haematoxylin and eosin stain, ×200X).
B. Strong membranous positivity of CD20 in DLBCL (CD20, ×100X).
C. Positive immunostaining of Pax-5 in DLBCL (Pax-5, ×200X).

Figure 1. Diffuse Large B Cell Lymphoma



A. Low power view showing nodular aggregates of lymphoma cells throughout the lymph node (Haematoxylin and eosin stain, ×40X). B, C and D. Positive immunoexpression of CD10, Bcl2 and Pax-5 in neoplastic follicles (CD 10, Bcl2 and Pax-5, ×40X).

Figure 2. Follicular lymphoma



A case of Acute lymphoblastic leukemia which showed negative immunoexpression of CD20 but positiveimmunohistochemical staining for Tdt and Pax 5 and thus was classified as B cell ALL. A.Haematoxylin and eosin stain, ×100X. B. Tdt, ×40X. C. Pax5, ×100X. D. CD20, ×100X.

Figure 3. B Cell-acute lymphoblastic leukemia.

All 7 (100%) cases of HL showed positivity of CD 30 in RS cells. CD15 was positive in 2 cases (28.5% cases).Pax-5 immunoexpression was seen in 5 out of 7 cases accounting for 71.4 % of cases. The expression came out to be statistically significant ($p<0.05$) with sensitivity of 77.7%.CD20 was however negative in all 7 cases (100%).All 4 (100%) cases of plasma cell neoplasm were CD138 and Kappa positive. None of the cases showed immunostaining for Pax-5. Two cases of lymphomas (1 case of SLL and 1 of NHL B cell type) showing infiltration of bone marrow were included in the study. Both the cases (100%) showed positive immunoreactivity for Pax-5. Thus, no difference was found in Pax-5 immunoexpression with respect to infiltration of bone marrow by lymphomas (Table 1)

DISCUSSION

Haematolymphoid malignancies are a major burden to afflicted patients both medically and financially. The diagnosis of haematolymphoid malignancies is based on a constellation of clinical, laboratory features, histomorphology, immunophenotyping and, where appropriate, molecular genetic analysis. Application of highly selective panel of immunostains, based on the histopathological impression of the tumor, can be extremely useful to narrow the diagnostic considerations as most lymphomas are substantially defined by their immunoprofile. In our present study, we attempted to correlate the expression of Pax-5 with the histological type of haematolymphoid malignancies and observed vast number of findings compatible with various studies and few findings contrary to previous studies. Pax-5 immunoexpression was seen in all cases of Non Hodgkin Lymphoma-B cell type whereas no expression was noticed in any case of Non Hodgkin Lymphoma-T cell type. Our results were in concordance with the study done by Desouki et al who found all cases of Diffuse large B-cell lymphoma (72/72) and all small B-cell lymphomas (5/5) to be positive for Pax-5 immunostaining and found absence of Pax-5 immunoexpression in all the cases of ALCL (n=22) (Desouki, 2010). Zhang et al studied the expression of Pax-5 in 70 B cell lymphomas and found 94.2% cases to be Pax-5 positive. However, Pax-5 was found negative in all T cell lymphomas (n=26) (Zhang, 2003) Krenacs et al demonstrated immunoexpression of Pax-5 in 81% (83/102) cases of B cell NHL but no Pax-5 immunoreactivity was seen in any case of T cell lymphomas (Krenacs, 1998).

All the 8 cases (100%) of B ALL in our study showed positive immunoexpression for Pax-5. However, positive immunostaining for CD20 was observed in only 5 cases (62.5%). The positive expression of Pax-5 in 3 cases of CD20 negative B ALL was statistically significant ($p<0.05$) and highly sensitive.Similar results were obtained in the study conducted by Dong et al where Pax-5 was invariably detected in 31/31 cases of pre B-ALL. Tiacci et al found no detectable Pax-5 immunoexpression in 50 T-cell ALLs (100%). However, blasts from 150 B-cell ALLs showed strong Pax-5 nuclear expression.[7]Contrary to our study, Zhang et al found expression of Pax-5 in 10 of the 14 B ALLs (72.4%), 3 of 6 T ALLs (50%), 13 of 26 AMLs (50%). The expression of Pax-5 in AML and T ALL in their study was explained by the fact that the expression of Pax-5 in hematopoietic progenitor had minimal effects on myeloid differentiation (Zhang, 2003). Our results in case of Hodgkin Lymphoma were in concordance with the study conducted by Desouki et al who found 36 of 41 (88%) cases positive for Pax-5 staining Fauceglia et al also observed positive Pax-5 immunoexpression in 60/70 (85.7%) HLs.[8]Torlakovic et al observed the expression of Pax-5 in 97% cases of classic Hodgkin lymphomas.[9]All (100%) plasma cell neoplasms included in our study were negative for Pax-5 immunostaining and the results were in concordance with the study of Zhang et al, Dong et al and Torlakovic et al. (2003) (Dong et al., 2008; Torlakovic, 2002) (Table 2). The diagnosis of various histological subtypes of haematolymphoid malignancies is challenging. Pax-5 is a valuable diagnostic marker for diagnosis of B-cell NHL, ALL and classical HL. The absence of Pax-5 expression in all cases of T-cell ALL and ALCL confirmed T-cell lineage and diagnosis in these cases.

Conclusion

Findings of our study demonstrated the relatively high specificity of Pax-5 expression for B cells. Even though there is an excellent correlation between CD20 and Pax-5 immunoexpression, Pax-5 exceeds the specificity and sensitivity of CD20 because of its earlier expression in B-cell differentiation and its ability to detect all committed B cells, including classical Hodgkin lymphoma. The presence of Pax-5 immunoexpression in Reed Sternberg cells in Hodgkin Lymphoma is helpful in differentiating certain cases of Anaplastic large cell lymphoma and other T cell lymphomas. Pax-5 is, thus, superior to CD20 in the diagnosis of pre-B acute lymphoblastic leukemia and classical Hodgkin lymphoma versus ALCL and NHL T cell type.Pax-5 is, thus, a valuable addition to the armamentarium of markers available for lymphoma subtyping.

REFERENCES

Desouki MM, Ginell R, Cherry D, Lazarchick J. 2010. PAX-5: A Valuable Immunohistochemical Marker in the Differential Diagnosis of Lymphoid Neoplasms. *J Clin Med Res.,* 8:84-8.

Dong HY, Browne P, Liu Z, Gangi M. 2008. PAX-5 is invariably expressed in B-cell lymphomas without plasma cell differentiation. *Histopathology.*53:278-87.

Fauceglia PM, Saxena R, Zhang S, Terracciano L, Sauter G, Chadhuri A, et al. 2007. Pax-5 immunoexpression in various types of benign and malignant tumours: a high-

throughput tissue microarray analysis. *J Clin Pathol.,* 60:709–14.

Ho C, Rodig SJ. 2015. Immunohistochemical markers in lymphoid malignancies: protein correlates of molecular alterations. *Semin Diagn Pathol*. 32:381-91.

Krenacs L, Himmelmann AW, Quintanilla-Martinez L, Fest T, Riva A, Wellmann A, et al., 1998. Transcription factor B-cell–specific activator protein (BSAP) is differentially expressed in B Cells and in subsets of B-Cell Lymphomas. Blood. 92:1308-16.

Rao S. 2010. Role of immunohistochemistry in lymphoma. *Indian J Med Paediatr Oncol*. 31:145-7.

Tiacci E, Pileri S, Orleth A, Pacini R, Tobarrini A, Frenguelli F, et al., 2004. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)–acute myelogenous leukemia. *Am Assoc Cancer Res.* 64:7399-404.

Torlakovic E, Torlakovic G, Nguyen PL, Brunning RD, Delabie J. 2002. The value of anti-pax-5 immunostaining in routinely fixed and paraffin-embedded sections: a novel pan pre-B and B-cell marker. *Am J Surg Pathol.,* 26:1343-50.

Zhang X, Lin Z, Kim I. 2003. Pax5 expression in Non-Hodgkin's Lymphomas and Acute Leukemias. *J Korean Med Sci.,* 18:804-8.

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