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The role of histology in predicting recurrence of type A thymomas: a clinicopathologic correlation of 23 cases

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Spindle cell thymomas (type A), as per the WHO definition, are benign tumors with an excellent prognosis. However, recent studies document aggressive behavior with local recurrences as well as extrathoracic metastases. More recently, Nonaka and Rosai have raised the question as to whether atypical features like cellular atypia, mitotic activity, necrosis, and vascular permeation could predict the adverse outcomes of these tumors. In an effort to address the 'atypia and outcome' issue of spindle cell thymomas, we analyzed our database of over 600 cases of thymic tumors to identify type A thymomas. The presence of histomorphological features like tumor size, nuclear shape and variability, mitotic rate, and presence/absence of necrosis were correlated with Masaoka stage, relapse/recurrence, and extrathoracic metastases. The study identified 23 patients of pure spindle cell thymomas (WHO type A) ranging in age from 40 to 88 years (median age, 54 years) and with male-to-female ratio of 1:0.9. Approximately 43% of the cases had recurrence or metastases during the followup period (average, 49 months). The presence of necrosis correlates with both relapse and extrathoracic metastases but not with the stage of diagnosis. However, none of the other clinical or histological features, including size, predominant nuclear shape, nuclear variability, and mitotic activity, were correlated with the outcome parameters, such as stage at diagnosis, presence or absence of relapse, and extrathoracic metastases. Histological atypia is fairly common in WHO type A thymomas. The presence of necrosis was associated with both locoregional and systemic disease. However, none of the other clinical or histological features correlated with aggressive behavior.
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Thymomas are rare tumors that arise from the thymus gland. The classification of these tumors is controversial and multiple classifications exist. In an effort to find common ground, the WHO schema was developed. This schema recognizes types A and AB and B1, B2, and B3 subtypes of thymomas in addition to rarer variants such as micronodular thymoma, metaplastic thymoma, or sclerosing thymoma. The WHO schema defines spindle cell thymomas (type A) as organotypic epithelial neoplasms lacking distinct lobulation and composed of bland, spindle/oval epithelial tumor cells with little to no lymphocytic infiltrates. They are classified as benign tumors that are associated with 100% survival rate at 5 and 10 years.[1–3] However, recent studies, by us amongst others, have challenged this concept.[4,5] Types A and AB tumors have been documented to have aggressive potential and the capability of spreading in or outside of the thoracic cavity.[4,6]

The recognition that some spindle cell thymomas might behave in an aggressive manner has led to a re-examination of the features seen in these tumors. More recently, a concept of 'atypical spindle cell/type A tumors' has been put forth by Nonaka and Rosai.[7] These tumors are characterized by the

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presence of one or more atypical features, such as the presence of cellular atypia (which is insufficient for the diagnosis of carcinoma), mitotic activity, tumor necrosis, and vascular permeation. It has been suggested that one or more of these features may be useful in predicting adverse outcomes of thymomas and could form the basis of further classification of type A tumors, akin to type B, into subtypes A1, A2, and A3.[7] These authors recommend further analysis of cases with followup data to examine the issue of atypia and outcomes.[7]

The aim of this study was to analyze the association, if any, that these parameters had in predicting behaviors/outcomes of type A thymomas. With this goal, we reviewed the clinical and pathologic features of a relatively large series of spindle cell/type A thymomas ($n=23$) on patients whose followup information was available. A correlation was attempted between histological features and stage of tumor and development of recurrence or extrathoracic metastasis.

Materials and methods

Slides from 600 cases of thymic tumors from the pathology department database at Indiana University School of Medicine were reviewed after obtaining approval from the Institutional Review Board. The cases included biopsy and excision specimens with hematoxylin and eosin-stained slides available. More than 100 spindle cell tumors were initially selected and subsequently subclassified accordingly by two authors (ITV and SB) using WHO guidelines (eg, type A, type AB, micronodular, or metaplastic thymoma). Cases that were discordant or difficult to classify were excluded. Only cases that fulfilled all the criteria for spindle cell thymoma (type A)—ie, had minor ($<10\%$) lymphocytes and lacked features of type B thymomas or carcinoma—were further analyzed.

Histological features evaluated in this study, included nuclear shape and variability, mitotic rate, and presence/absence of necrosis. Nuclear features were classified into four types: (1) bland spindled nuclei with dispersed nuclear chromatin and inconspicuous nucleoli, (2) bland round to oval nuclei with vesicular nuclear chromatin; small nucleoli may be visible, (3) large nuclei with open chromatin and big nucleoli, and (4) frankly hyperchromatic anaplastic nuclei. Cases with type 3 or type 4 nuclear features were considered in the range of carcinoma and excluded from the study. In addition, nuclear characteristics like size, contour, chromatin features, and presence of nucleoli were also analyzed. Nuclear size was scored in comparison with the size of a red blood cell and classified large (greater than or equal to twice the size of a red blood cell) and small (less than twice the size of a red blood cell). Nuclear contour was classified as round (smooth) and irregular (for the nuclei with shrunken contour). Nuclear chromatin was classified as granular (coarse) or as 'fine' with homogenous distribution. The presence/absence of nucleoli was classified as 'no nucleoli,' 'inconspicuous nucleoli,' or 'small nucleoli.' Mitotic activity was evaluated in 10 consecutive high power fields (hpf) and classified as either 0–1 or >1 mitosis/hpf. Presence/absence of necrosis was recorded based on gross and/or microscopic findings.

Tumors size, clinical data, and followup information were obtained from the patients' charts or from referral institution and correlated with histological data obtained. The clinical parameters correlated included Masaoka stage, presence of relapse/recurrence, and extrathoracic metastases.

Results

Clinical Findings

The median age of the 23 cases of type A thymomas was 54 years (range, 40–88 years) with a male-to-female ratio of 1:0.9 (Table 1). The vast majority presented with chest mass-related symptoms. The tumor stage at the time of diagnosis was stage I or II in 12 cases, stage III in six cases, stage IVA in three cases, and IVB in two cases.

Treatment Details

All 23 patients underwent surgery; four had received prior neoadjuvant chemotherapy. Eight patients received postoperative radiotherapy and five received adjuvant chemotherapy.

Followup Data

The average followup duration for all patients was $\sim$49 months (4 years). Of the 23 patients, two patients had metastases at diagnosis (stage IVB) (Table 1). Pleural involvement was the most common site of local recurrence. Although lung was believed to be involved in many cases, as documented by imaging, differentiation from pleural involvement was not always possible. Six patients developed distant metastases 7 to 107 months after initial diagnosis. The sites included liver (four cases), neck (two cases), and bone (one case). Of note, the only one patient who had high mitotic activity (more than 10 mitoses/10 hpf) developed liver metastases 7 months after diagnosis and died after 18 months in spite of aggressive combination therapy.

Margin results: In the current series, five patients had close margins (<1 mm) and three cases had positive margins; of these eight patients, only one developed metastatic disease. In contrast, 9 of the 15 patients with negative margins went on to develop recurrences and/or metastases.

Table 1 Clinicopathological characteristics of patients with spindle cell thymomas (WHO type A)

No	*Sex*	*Age*	*Stage at DX*	*Primary treatment*	*Margin status after treatment*	*Site of metastases*	*Time to metastases (months)*	*Duration of followup (months)*
1	M	52	IVA	S + R	Neg	Lung	107	103
2	F	88	III	S + C	Neg	Neck	18	68
3	F	65	II	S	1 mm	None	No	2
4	F	53	IVA	S + C + R	Pos	Liver	7	18[a]
5	M	76	I/II	S	Neg	None	No	4
6	M	52	I/II	S	Neg	None	No	92
7	F	76	III	S + C	Neg	Liver	10	24
8	M	53	I/II	S + C + R	Neg	Liver	47	184
9	F	50	I	S	Neg	None	No	6
10	M	41	III	NACT + S + R	1 mm	None	No	61
11	M	55	II	S	1 mm	None	No	6
12	F	43	IVB	NACT + S + R	Neg	Lung/liver/ bones	N/A	91
13	M	49	IVB	NACT + S + R	Neg	Neck/lung	N/A	57
14	F	40	II	S	Neg	Lung	10	37
15	F	42	I/II	S	Neg	None	23	23
16	F	78	II	S + C	Neg	Pleura	91	95
17	M	48	III	S	1 mm	None	No	1
18	M	68	II	S + R	Neg	Lung	15	30
19	F	54	I	NACT + S + R	Neg	None	No	60
20	F	74	IVA	S	Pos	Pleura	No	26
21	F	62	III	S	Pos	None	No	36
22	M	80	III	S	1 mm	None	No	19
23	M	71	I	S	Neg	None	No	85

Abbreviations: C, adjuvant chemotherapy; NACT, neoadjuvant chemotherapy; S, surgery; R, radiotherapy; N/A, not applicable.
[a]Death due to disease.

Pathological Features

Gross examination. Gross findings, where possible, were obtained from the patients' pathology reports. The tumors weighed from 31–1230 g and ranged in size from 2.8–20 cm. At the time of surgery, nine tumors were documented as encapsulated and five as infiltrative. Primary tumor was identified as multiple tumor nodules in four cases. Necrosis had been noted on gross examination in two cases.

Histological findings. The histological findings of all 23 cases included in this study are summarized in Table 2. Although all slides from the tumor had been reviewed at some stage, the number of slides available for review for this study per case ranged from 1–20 (average number, four slides per case). The tumor in all cases was composed of spindle cells arranged in short fascicles with few or no admixed lymphocytes. The nuclei in the majority of cases were round to oval. In two cases, the nuclei were elongated and fusiform, and a mixed pattern was noted in additional two cases. Nucleoli were not identified in nine cases; small inconspicuous nucleoli were identified in 12 cases, these were prominent in two cases. As cases with marked nuclear atypia were excluded from the study, most cases included in the series did not exhibit significant pleomorphism. In 16 out of 23 cases, nuclear size was greater than two red blood cells and the nuclear contour was round in 17 cases (Figure 1). Nuclear chromatin was coarse and granular in 17 cases and finely distributed in six cases (Figure 2).

Mitotic activity was generally very low (0–1 mitoses/hpf), although in one case it was prominent (Figure 3). Microscopic foci of necrosis were found in four cases (Figure 4). Six cases (25%) presented microcystic spaces while seven cases (29%) presented microhemorrhages. Fibrous septa were identified within the tumor in 11 cases (46%) and in one case residual Hassall corpuscles were present. The surgical margins were reported as negative in 15 cases, close (<1 mm) in five cases, and positive in three case.

Correlation with Patient Outcomes

Table 2 shows the correlation of histomorphological features with recurrence and metastases. Of the several features analyzed, only the presence of necrosis correlated with adverse outcomes. Interestingly, the only case with greater than 10 mitoses/10 hpf developed liver metastases and died within 2 years of diagnosis. However, none of the features showed statistically significant association with outcomes.

Discussion

WHO type A thymomas have, of late, become a topic of interest. This is due in part to the documentation that their clinical course is not as benign as initially

Table 2 Correlation of histomorphologic features with stage, relapse, and extrathoracic metastases

Feature	*Feature*	*Stage*			*Relapse*			*Metastases*		
		I/II	*III/IV*	P *value*	+	−	P *value*	+	−	P *value*
Tumor size	>5 cm	3	5	NS	3	5	NS	0	8	NS
	<5 cm	1	2		1	2		1	2	
	Unknown	6	6		6	6		5	7	
Necrosis	Present	2	2	NS	4	0	0.02	3	1	0.04
	Absent	10	9		6	13		3	16	
Nuclear shape	Oval	9	11	NS	8	12	NS	6	14	NS
	Spindle	3	0		2	1		0	3	
Nuclear variability	Present	10	9	NS	8	11	NS	5	14	NS
	Absent	2	2		2	2		1	3	
Mitotic activity	High	0	1	NS	1	0	NS	1	0	NS
	Low	12	10		9	13		5	17	
Nuclear size	<2 Red blood cells	5	2	NS	3	4	NS	1	6	NS
	≥2 Red blood cells	7	9		7	9		5	11	
Nuclear contour	Regular	7	10	NS	8	9	NS	5	12	NS
	Irregular	5	1		2	4		1	5	
Chromatin	Granular	11	6	NS	8	9	NS	4	13	NS
	Fine	1	5		2	4		2	4	
Nucleoli	No	5	4	NS	5	4	NS	4	5	NS
	inconspicuous	7	5		4	8		1	11	
	small	1	1		1	1		1	1	

Abbreviation: NS, not significant.

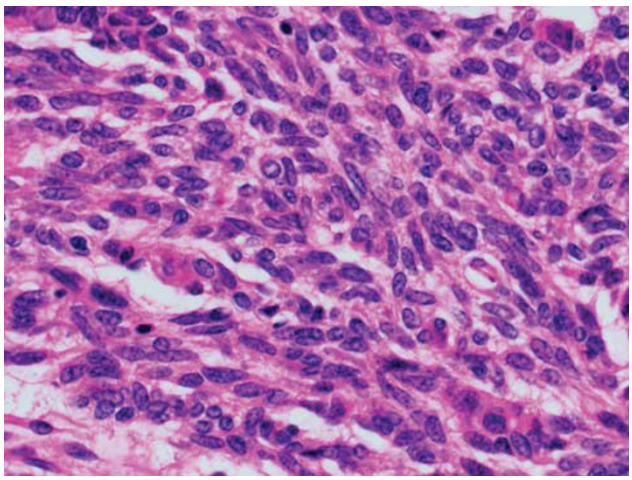

Figure 1 Spindle cell thymoma (WHO type A) with bland spindle shaped nuclei. Note the lack of nuclear pleomorphism or mitotic activity.

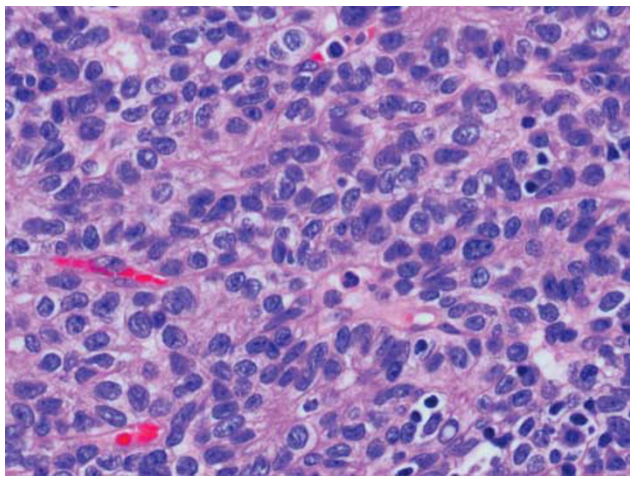

Figure 2 Spindle cell thymoma (WHO type A) with round to oval plump nuclei. Note that this case shows some nuclear atypia in the form of open chromatin and small pink nucleoli.

presumed. This was partly a result of overreliance on overall survival rather than metastases as an endpoint for assessing behavior. Several studies have documented invasive growth and even metastases in tumors of this subtype.[4–6] This realization has prompted a closer look at the morphological features that could be associated with adverse outcomes. Nonaka and Rosai[7] published a series of 13 cases of type A thymomas showing atypical features. These features included increased mitotic activity, the presence of mild to moderate nuclear atypia, and/or scattered foci of necrosis. The tumors described in this series ranged from 2–8 cm, exhibited mitotic activity in the range of 5–13 mitoses/10 hpf, and foci of necrosis were identified in six tumors. Although one of these patients had stage IV disease at diagnosis, none of the six patients developed recurrence or metastases in the followup period. The issues raised in this manuscript, include whether morphological atypia is associated

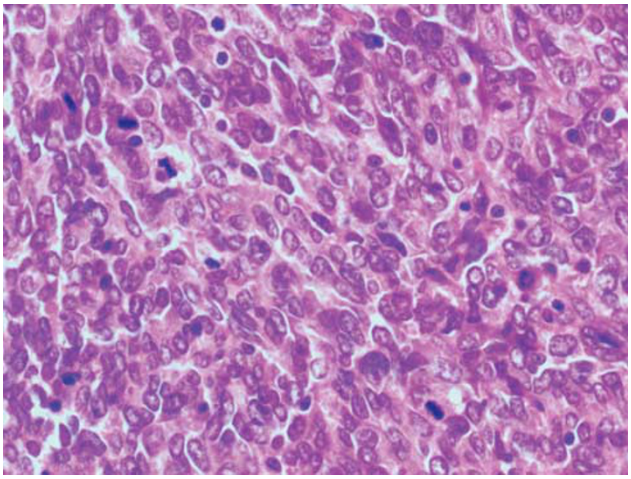

Figure 3 Spindle cell thymoma (WHO type A) with high mitotic activity. Note the lack of nuclear pleomorphism in spite of the high mitotic activity.

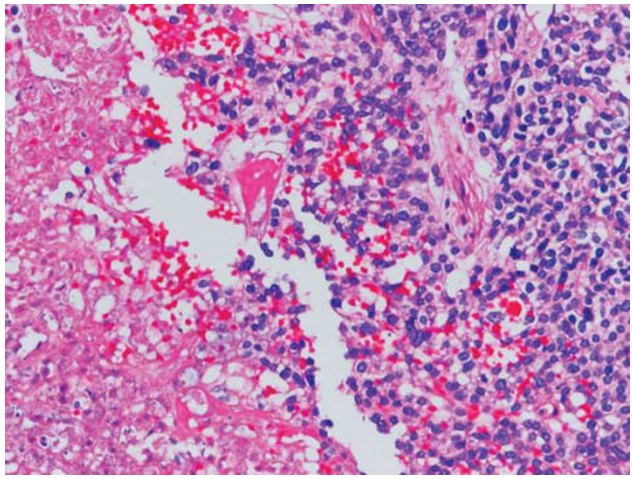

Figure 4 Spindle cell thymoma (WHO type A) with small foci of necrosis.

with adverse outcomes and whether this could be used to further subclassify type A thymomas into A1, A2, and A3—bringing them on par with the type B thymomas.

The current study seeks to question the relevance of atypical features in predicting outcomes of type A thymomas. The series is composed of a significantly larger number of cases with recurrence; this reflects an institutional bias. Indiana University Simon Cancer Center (IUSCC) is an established referral center for the treatment of patients with advanced thymomas, resulting in overrepresentation of more advanced cases of thymomas and thymic carcinomas. However, this patient population provides for an ideal test series for the evaluation of features associated with recurrence and/or metastases in relatively indolent forms of thymomas such as type A. Most of the cases had primary tumor surgery at referring institutions before being seen at IUSCC; this limits the assessment of some important parameters such as type of surgery and number of blocks/slides (for details see Moran and Suster[8,9]) but provides for a more real world situation.

Tumor stage and completeness of excision are important determinants of adjuvant therapy in thymomas. The extent of surgery has been documented to impact the likelihood of recurrence in thymomas.[10] It was often stated in older literature that subclassifying noninvasive carcinoma by histology was unnecessary. In the current series, recurrences and metastases were seen irrespective of margin status. On the basis of this data, it appears that the importance of margin status in assessing the biology of thymomas might have been overstated. These observations are similar to those reported by Moran *et al.*[6] It needs to be noted that Masaoka stage and completeness of excision have been traditionally correlated with overall survival.[11] This leads to the false impression of benignity of thymomas. Even after the development of metastases, patients with thymomas can survive for decades.[10]

Pan *et al*[12] have classified type A thymomas into two subtypes, short spindled and long spindled variants in addition to micronodular variants. The latter were not included in the current series. In their series of 38 cases, the short spindle variant accounted for 57% of cases. We also observed two distinct morphological types in our series with short spindled variant accounting for most of the cases. The long spindled variant was invariably associated with slender fusiform nuclei and showed minimal to no atypia. Of the four cases with these features in our series, two showed relapse and one showed extrathoracic metastases.

Nuclear shape and presence of small nucleoli did not appear to be a significant predictor of behavior in the current series. The presence of atypia did not show an association with higher tumor stage. Conversely, cases that lacked nuclear pleomorphism were still associated with adverse outcomes. Although it must be noted, cases with prominent pleomorphism were excluded from the current series. This was done in part to avoid contamination of the cases with spindle cell variants of type B3 thymomas and sarcomatoid thymic carcinomas. The development of specific molecular markers for thymomas will enable recognition of cases with prominent nuclear atypia with confidence. But until such time, we have refrained from diagnosing cases with prominent nuclear anaplasia as type A thymomas.

Type A thymomas, in general, are not characterized by prominent mitotic activity.[4,6] Recently, prominent mitotic activity ranging from 5–13 mitoses/10 hpf in type A thymomas was reported by Nonaka and Rosai.[7] Although none of their patients developed recurrent disease, the long-term followup was available in only 5 of 13 patients. In the current series, only one patient had prominent mitotic activity (>10 mitoses/10 hpf); this patient developed metastases within 7 months of initial diagnosis and died of

progressive disease in spite of aggressive combination therapies. Spindle cell carcinomas can exhibit significant mitotic activity. However, as defined by Suster and Moran[13] these lesions at least focally showed 'frank' features of malignancy in the form of 'an overtly malignant population of spindle cells characterized by large, hyperchromatic nuclei with prominent nucleoli and frequent mitotic figures.' Cases with these features were excluded from the current study. None of the cases described herein demonstrated significant cytologic atypia.

The presence of necrosis in type A thymomas is rare but described by Nonaka and Rosai[7] as a feature of atypia. Scattered foci of necrosis were observed in four cases (including two cases with grossly apparent necrosis); these were not related to prior (neoadjuvant) therapy. These tumors showed aggressive behavior with relapses being noted in all cases and with metastases in three. This association was significant for relapse ($P = 0.02$) and metastases ($P = 0.04$) but not for stage at diagnosis.

In conclusion, the current series confirmed some of the well-described attributes of type A thymomas, including the rarity of necrosis, atypia, and high mitotic activity. This was unusual even in cases that were associated with recurrence/metastases. The analysis showed that aggressive behavior was not associated with specific cytomorphological features or presence of prominent mitotic activity. The presence of tumoral necrosis was associated with adverse outcomes.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Muller-Hermelink HK, Marx A. Pathological aspects of malignant and benign thymic disorders. Ann Med 1999;31(Suppl 2):5–14.
2 Okumura M, Miyoshi S, Fujii Y, *et al.* Clinical and functional significance of WHO classification on human thymic epithelial neoplasms: a study of 146 consecutive tumors. Am J Surg Pathol 2001;25: 103–110.
3 Okumura M, Ohta M, Tateyama H, *et al.* The World Health Organization histologic classification system reflects the oncologic behavior of thymoma: a clinical study of 273 patients. Cancer 2002;94:624–632.
4 Jain RK, Mehta RJ, Henley JD, *et al.* WHO types A and AB thymomas: not always benign. Mod Pathol 2010;23:1641–1649.
5 Vladislav T, Jain RK, Alvarez R, *et al.* Extrathoracic metastases of thymic origin: a review of 35 cases. Mod Pathol 2012;25:370–377.
6 Moran CA, Kalhor N, Suster S. Invasive spindle cell thymomas (WHO Type A): a clinicopathologic correlation of 41 cases. Am J Clin Pathol 2010;134: 793–798.
7 Nonaka D, Rosai J. Is there a spectrum of cytologic atypia in type a thymomas analogous to that seen in type B thymomas? A pilot study of 13 cases. Am J Surg Pathol 2012;36:889–894.
8 Moran CA, Suster S. The World Health Organization (WHO) histologic classification of thymomas: a reanalysis. Curr Treat Options Oncol 2008;9:288–299.
9 Moran CA, Suster S. Thymic neuroendocrine carcinomas with combined features ranging from well-differentiated (carcinoid) to small cell carcinoma. A clinicopathologic and immunohistochemical study of 11 cases. Am J Clin Pathol 2000;113:345–350.
10 Venuta F, Rendina EA, Anile M, *et al.* Thymoma and thymic carcinoma. Gen Thorac Cardiovasc Surg 2012; 60:1–12.
11 Masaoka A, Monden Y, Nakahara K, *et al.* Follow-up study of thymomas with special reference to their clinical stages. Cancer 1981;48:2485–2492.
12 Pan CC, Chen WY, Chiang H. Spindle cell and mixed spindle/lymphocytic thymomas: an integrated clinicopathologic and immunohistochemical study of 81 cases. Am J Surg Pathol 2001;25:111–120.
13 Suster S, Moran CA. Spindle cell thymic carcinoma: clinicopathologic and immunohistochemical study of a distinctive variant of primary thymic epithelial neoplasm. Am J Surg Pathol 1999;23: 691–700.