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Tumour volume and macroscopic growth pattern of bronchogenic carcinoma

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Summary. 126 resected malignant lung tumours were cut into serial sections. The tumour volume, the pTN-stage and the macroscopic growth pattern were computed. The average tumour volume was found to be 28.5–35.8 cm^3 (confidence limits, $p \geqq 95\%$) and to be independent of the major cell type of the carcinomas. The cell types grow in different macroscopically distinguishable tumour growth patterns: epidermoid carcinomas grow mostly in bizarre finger-like shapes and small cell anaplastic and adenocarcinomas more frequently in ball-like shapes. The different growth patterns depend on the cell type, the cellular response of the host tissue and the tumour volume. Due to irregular growth pattern the tumour volume computed by X-ray diameter is likely to be overestimated by 50%–300%.

Key words: Bronchogenic carcinoma, Tumour volume, Macroscopic growth, Cellular response

Introduction

It is necessary to improve our knowledge of the relationship of growth patterns and tumour volume of lung carcinoma in surgically treatable patients. Although the mechanism of neoplastic transformation is not well understood, clinically useful information may be obtained by studying the mode of growth of tumours.

The pre-surgical TNM-staging of bronchogenic carcinoma is based on the maximum diameter of the tumour, and on additional local conditions (inflammation, tumour distance to the carina, etc.) (UICC 1979). However, the maximum tumour diameter in surgical specimens cannot be computed without knowing the geometric centre of the carcinoma.

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The maximum tumour diameter is a derivative of the really important variables, the number of the tumour cells, or the tumour volume. The size and localization of the tumour define the mode of surgical treatment (Berrino 1971; Buell 1969; Mountain 1977; Vogt-Moykopf 1981), the post-surgical therapy, the probability of lymph node involvement, etc. (Matthews 1983; Underwood 1974). Therefore, exact measurement of tumour volume, tumour growth conditions, etc. seem to be a task for pathology. A method of reconstruction of malignant tumours was first introduced by Petersen 1902, who used serial sections of skin carcinoma and wax models. His models, however, were limited to the microscopic appearance of epidermoid tumors. No application was made to the problems of tumour volume. To our knowledge his technique has not been repeated by other authors.

In this paper we present a technique for measuring tumour volume. The macroscopic shape of the tumour can be reconstructed and tumour-cell-growth centers can be identified.

Materials and methods

126 surgical specimens (lobes and lungs of operated patients with a tumour of the lower respiratory tract) received by the Institute of Pathology, University of Heidelberg, were fixed as follows: With moderate pressure (50 mmHg) air was blown into the major bronchi. A solution composed of acetone (60%) and buffered formalin (40%) was instilled through the bronchi during expansion of the lung tissue. The specimens were allowed to fix for additional 24 h and were then cuto into sagittal slices 5 mm thick. One slice was used for histological examination. It was stained with HE, PAS, and Masson's stain. The other slices were dehydrated (acetone) and impregnated with polymers according to the method described by von Hagens (1979). The acetone was evaporated in a vacuum chamber and replaced by polyester resin. After impregnation the slices were then packed between glass plates and hardened under pressure at a temperature of 50° C (von Hagens 1979; 1982).

Impregnation with polyester resin changes the refraction index of the slices. The transparent slices were examined with a conventional microscope (magnification 60:1) and the tumour boundary was marked on the slices. The volume of the tumour was computed by measuring the area of each tumour slice, multiplying the thickness of the slice and summing the tumour volumes of the individual slices. The detailed procedure is described elsewhere (Kayser et al. 1983).

Macroscopic reconstruction of the tumour was performed in the following manner: The geometric centre of each tumour slice was computed using a graphic tablet connected to a TEKTRONIX 4051 computer. A perspective projection onto an artificial coordinate system was performed by setting the centre of each slice onto a 45° line. By following the sequence and the thickness of the individual slices, the 3-dimensional surface boundary of the tumour was drawn. Only the non-overlapping portions of the slices are shown. Consistent with the cutting procedure the angle of view displays the proximal part of the tumour (proximal to the hilus). Over-estimation of the theoretical tumour volume was computed as follows:

$$O = (V_T - V_E) \times 100 / V_T$$

O = over-estimation in percent
V_T = theoretical tumour volume computed using the maximum diameter and a spheroid shape
V_E = actual tumour volume

The pT-stage and the pN-stage were classified according to the rules of the UICC 1979.

The "cellular response" of the host tissue was obtained by grading the number of lymphocytes, plasma cells and macrophages at the tumour boundary or inside the tumour. Large histological specimens containing a complete cross section of the tumour were analysed.

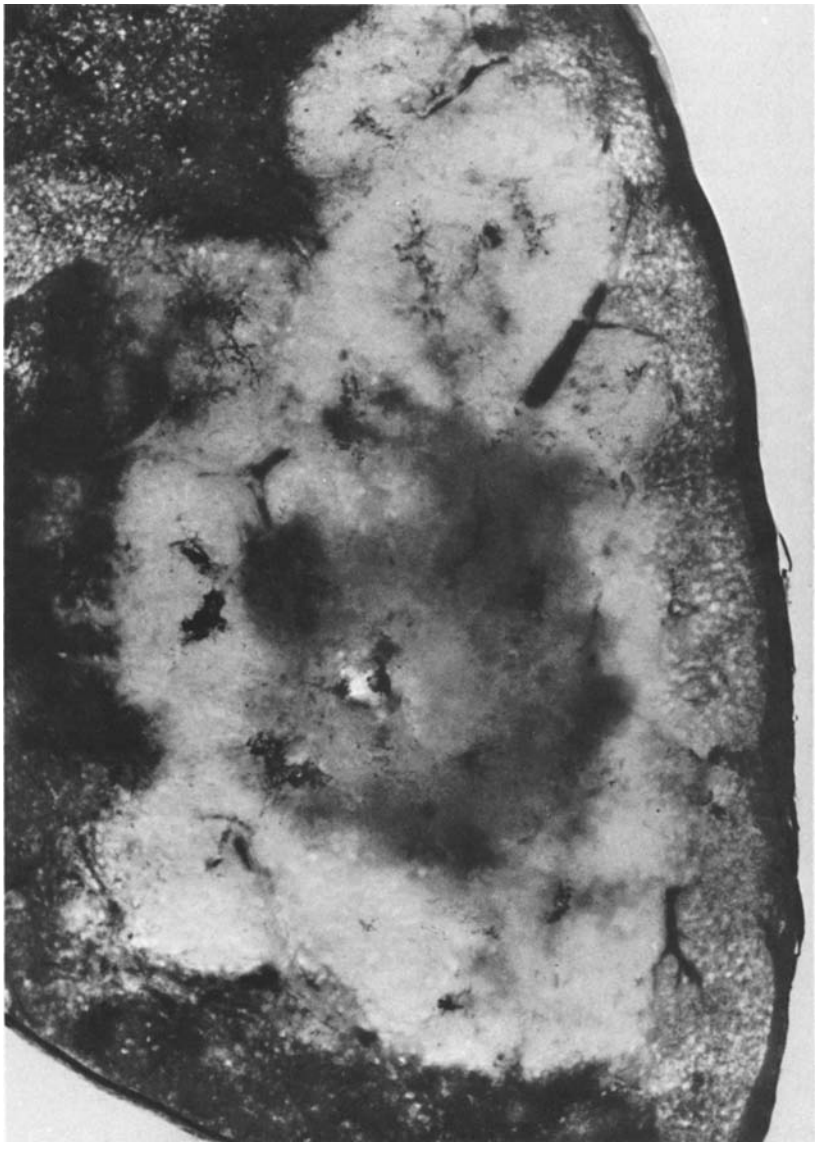

Fig. 1. Polymer-impregnated lung slice showing a central moderately differentiated adenocarcinoma with multiple proliferation centers and central necrosis.

Results

Accuracy of the method

A resin-impregnated lung tissue slice of a moderately differentiated adenocarcinoma with multiple proliferation centres is shown in Fig. 1. Clear separation of the tumour and the surrounding lung tissue can be noted. Resin

embedding allows examination of the tumour boundaries of the 5 mm thick slices with a conventional microscope at moderate magnification. Possible infiltration of tumours can be detected and was taken taken into account. Tumour volume is computed by using an iterative method where the probable error of computation of tumour volume depends on the number and the thickness of the lung slices. Since most of the tumours had a maximum diameter of 3 cm or more, at least 6 slices were obtained for every lesion. This represents a maximum systematic error of about 10%, if all errors were in the same direction. According to the iterative method the computed tumour volume is larger than the actual one. The shrinkage of the tumour and lung tissue due to fixation is about 5%–10%. Thickness of slices is measured with an accuracy exceeding 95% (error $<5\%$); the total error of the procedure, assuming undirectional error, can be estimated at 10%–15% depending on the tumour size. It must be pointed out, however, that except for shrinkage, the error is likely to be random and hence less than those values which assume undirectionality.

Tumour volume and cell type

Tumour volume is an important variable in prognosis. Several authors have reported a close relationship of survival rate to maximum diameter of the tumour measurement by X-ray (BERRINO et al. 1971; Buell 1969; Freise et al. 1977; Lockich 1972; Soorae et al. 1977).

The TNM- and pTNM-classification is based on the maximum diameter of the tumour. One approach of this study was the computation of the actual tumour volume, values of which, grouped according to cell type are shown in Table 1. No statistically significant differences among the four major cell types could be obtained. The smallest tumours were found to be metastases and carcinoids. Sarcoma showed the largest tumour volume. Unfortunately the small cell carcinoma could not be classified with respect

Table 1. Average volume and maximum diameter of 126 lung tumors grouped according to cell type

Cell type	*N*	Tumour volume		
		mean (cm^3)	Confidence-limits (95%)	Max. diameter mean (cm)
Epidermoid	56	27.5	23.4–32.5	4.8
Adeno	27	38.8	31.7–47.5	5.1
Large cell	13	35.5	26.3–47.9	4.7
Small cell	12	35.9	27.4–47.0	5.1
Metastasis	10	22.2	14.3–34.5	5.4
Carcinoid	4	14.3	10.9–18.8	3.5
Sarcoma	4	225.0	72.4–700.5	9.4
Total	126	32.1	28.8–35.8	5.0

Table 2. Average tumour volume and pT-stage (108 cases) and pN-stage (104 cases) of primary lung carcinoma (mean and confidence limits (95%))

	N	Mean	(95%) Confidence limits
pT-Stage			
pT1	19	7.8	6.5– 9.3
pT2	71	33.5	30.3– 37.2
pT3	18	115.5	99.0–134.8
pN-Stage			
pN0	48	19.9	16.8– 23.5
pN1	47	45.0	39.0– 51.8
pN2	9	45.1	30.4– 67.1

to its neuro-endocrine activity (Bombesin, Neuron-Specific-Enolase) due to the lack of availability of monoclonal antibodies. All carcinoids were found to be typical (benign) carcinoids, localized in a central major bronchus.

Tumor volume and pTN-stage

By definition the pT-stage depends on the tumour volume. The majority of the cases (66%) were operated on at the pT2-stage. The average tumour volume for the pTN-stage is shown in Table 2. Since no significant differences in the prognostic value of either pT1- or pT2-stage was obtained in our data, tumours with an average volume of 37.2 cm^3 or less were regarded as similar with respect to the prognosis of the patients (Kayser et al. 1983).

The probability of lymph node infiltration (pN1, pN2) increased with increasing tumour volume. This is in agreement with published clinical data (Berrino et al. 1971; Buell 1969; Giedl et al. 1983). No remarkable differences between the pN1- and pN2-stage could be found, but great differences between pNO-and the other pN-stages were observed (Table 2).

This is consistent with survival times of the patients in this study and those of a retrospective study carried out by us in 1979 (Kayser et al. 1982). In that particular study the survival time of patients in the pN1-stage did not differ from that of patients in the pN2-stage.

Tumour volume and X-ray

The amount of over-estimation of the tumour volume based on maximum X-ray diameter is shown in Fig. 2. The over-estimation of the actual volume increases with increasing maximum diameter. For larger tumours an over-estimation of 200%–400% can be noted, i.e. only 20%–30% of the theoretical tumour volume is actually occupied by tumour cells. This fact may explain the failure of tumour kinetic models based upon X-ray diameter only (Kayser et al. 1982).

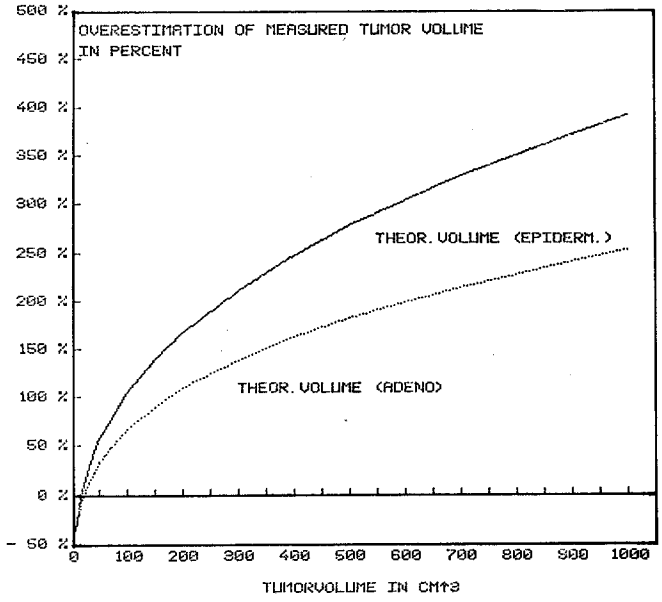

Fig. 2. Over-estimation of actual tumour volume in comparison to the theoretical volume computed by maximum X-ray diameter and assumed spheroidal shape

Macroscopic reconstruction

Depending on the macroscopic tumour shape, four different categories of growth could be distinguished:

1. Tumours growing in "finger-like" formations with irregular bizzare tumour cell propagation into the lung tissue. An example of this growth pattern is shown in Fig. 3. The histology of this case showed a moderately differentiated epidermoid carcinoma.
2. Tumours growing in a spheroidal shape without any preferred direction of cell propagation into the lung tissue. A central, moderately differentiated adenocarcinoma showing this macroscopic growth pattern is given in Fig. 4.
3. Tumours growing in ellipsoid shapes. The tumours were grouped into this category if the ratio of the maximum and the minimum diameter was calculated to be 2:1 or higher. An example of this category is shown in Fig. 5. The tumour shown here was found to be a small cell anaplastic carcinoma.
4. Tumours which could not easily be classified into the above groups. Most of these tumours grew in ellipsoidal or spheroidal shapes with several prominent proliferation centres at the boundary. A tumour of this category, a large cell undifferentiated carcinoma is shown in Fig. 6.

The statistical data regarding macroscopic tumour classification and cell type are given in Table 3. The macroscopic growth pattern is dependent

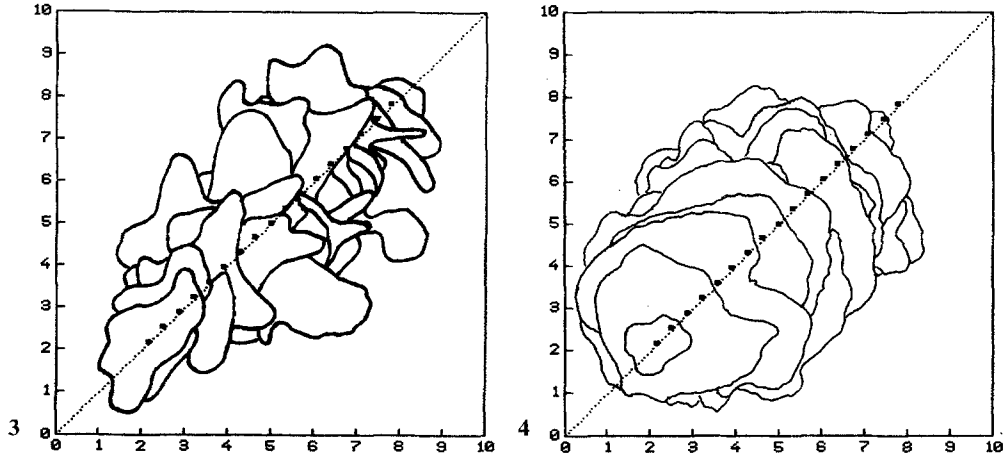

Fig. 3. Bizarre macroscopic growth of a central, moderately differentiated epidermoid carcinoma (pT2, N1). Direction of view from the hilus to the distal ventral lung.

Fig. 4. Spheroid macroscopic growth of a central, moderately differentiated adenocarcinoma (pT2, N1). Direction of view from the hilus to the distal ventral lung.

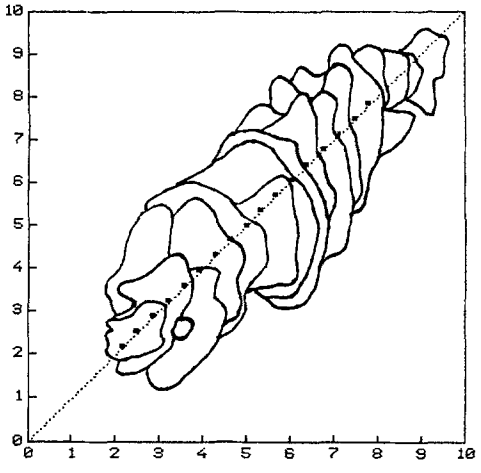

Fig. 5. Ellipsoid macroscopic growth of a central small-cell anaplastic carcinoma, oat-cell type (pT2, N2). Direction of view from the hilus to the ventral lung.

on the cell type. Only a minority of the tumours showed a generally spheroidal growth pattern. The majority were adenocarcinomas or metastases. In epidermoid carcinomas the expansion of the tumour cells seems to depend on pre-existing structures of the host tissue, i.e. bronchi or vessels. This does not hold true for small cell anaplastic or adenocarcinomas. In some cases large cell undifferentiated carcinomas show a growth pattern similar to that of epidermoid carcinomas.

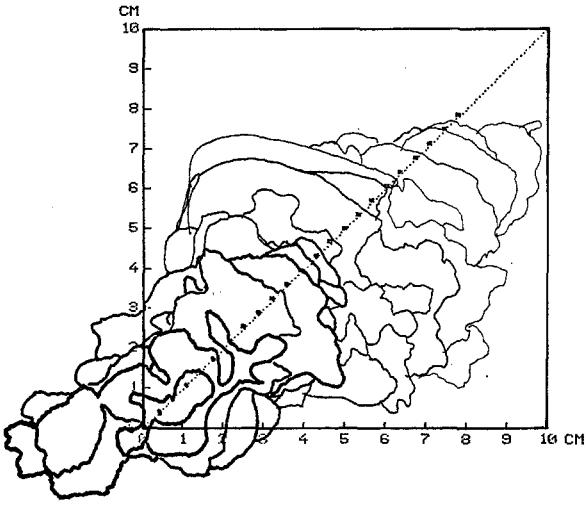

Fig. 6. Mixed (ellipsoidal and bizarre) macroscopic growth of a central undifferentiated large cell carcinoma (pT3, N1). Direction of view from the hilus to the ventral lung.

Table 3. Macroscopic tumour growth and cell type in 126 lung tumour cases

Tumour growth	Cell type													
	Metastasis		Epidermoid		Adeno		Large cell		Small cell		Carcinoid		Sarcoma	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Bizarre	1	10	33	59	0	0	2	15	0	0	0	0	0	0
Spheroid	3	30	3	5	20	74	0	0	0	0	0	0	0	0
Ellipsoid	2	20	1	2	0	0	3	23	8	67	4	100	2	50
Mixed	4	40	19	34	7	26	8	62	4	33	0	0	2	50
Total	10	100	56	100	27	100	13	100	12	100	4	100	4	100

Host tissue response

Several indications have been reported that malignant tumours can stimulate the immunologically responsive system either by tumour-associated surface antigens (TASA) (Herberman et al. 1979; Herberman 1982; Poste et al. 1982; Schirrmacher et al. 1979) or by destruction of host tissue and reactive inflammation. Our data regarding the macroscopic growth patterns and the associated inflammatory infiltration of the tumours are given in Table 4. About 50% of the tumours showing a bizarre growth pattern (group I) were infiltrated by lymphocytes, plasma cells and macrophages. Only 10%–20% of cases with dense inflammatory infiltrations (tumours and boundary) were associated with an ellipsoidal shape (group III). No or few inflammatory cells were associated with a macroscopic bizarre shape in about 30% of the cases. These data indicate a relationship of macroscopic-growth pattern to cell type and reactive inflammation in the host tissue,

Table 4. Macroscopic growth and immune response in 126 lung tumour cases

Tumour growth Pattern	Tumour boundary Immune response							
	None		Scattered lymphocytes		Dense lymphocytes		Tumour infiltration	
	N	%	*N*	%	*N*	%	*N*	%
Bizarre	6	15	5	16	13	43	12	52
Spheroid	6	15	10	31	6	20	4	17
Ellipsoid	13	32	3	9	3	10	1	4
Mixed	16	39	14	44	8	27	6	26
Total	41	100	32	100	30	100	23	100

in agreement with other authors (DiPaola et al. 1977; Takakura et al. 1983). Whether this inflammation is due to tumour related products (TASA) cannot be determined. The fact that most of the cells were plasma cells indicates that the inflammatory infiltrations may be partly related to damage of the lung. Watanabe et al. 1983 found a high number of precursor T-cell subpopulations (helper and suppressor cells) in the majority of their bronchial carcinomas, but only a minority of natural killer cells. The inflammatory cells were located in the tumour stroma. These data indicate that in addition a direct relationship between the inflammatory response of the host tissue and the tumour growth may exist (Herberman et al. 1979; Vanky et al. 1983).

Discussion

The spread of malignant tumours in human tissue is not well understood. In lung cancer, clinical investigations regarding tumour propagation seem to be fairly easy using X-ray examinations and computed tomography. The data presented give an idea of the magnitude of errors associated with orthographic projection of lung tumours. Although the tumour volume is quite small at the time of surgical treatment a significant number of tumours show an irregular bizarre propagation into the host tissue. Whether this irregular growth is caused by different nutrition (vascularisation), by different tumour cell sub-populations or by immune defense mechanisms of the host tissue still remains an open question. In our opinion, the variables play a part in the propagation of tumour cells. As shown by Müller-Schwickerath 1978, vascularisation of bronchogenic carcinomas is not uniformly distributed. According to their data several ball-like vascularisation centres combined with new bronchial capillaries are to be found either at the center or on the surface of tumours. At an early stage (tumour diameter less than 2 cm), proliferation of new vessels with a straight hilufugal orientation took place. This fact may explain the ellipsoidal shape of some bronchogenic

carcinomas. At the later stage almost random orientation of the vessels and new formation of broncho-pulmonary-arteries was observed. This was especially true in large tumours with a diameter of 6 cm or more (Müller-Schwickerath 1978). This phenomenon is consistent with the macroscopic growth pattern seen in the majority of epidermoid carcinomas and sometimes in large cell anaplastic carcinomas.

However, strong indication of tumour cell heterogeneity of metastases into the human lung (Mattern et al. 1981; Talmade 1983; Underwood 1974) and in animal experiments (Poste et al. 1982) has been reported. Lung carcinoma cells transplanted into nude mice show varying proliferative activity and multiple tumor cell sub-populations (Mattern et al. 1981). Assuming different growth velocities in different tumour cell subpopulations the observation of different growth centres in bronchogenic carcinomas tend to support these findings.

Conclusion

The data presented must be regarded as an early step toward an increase in our knowledge of tumour growth conditions in bronchogenic carcinoma. The method described is not only useful in examining human lung tumours but is also helpful in animal experiments. Some authors suggest differences in the biological behaviour between spontaneous tumours of inbred rodents and experimentally tumours caused by carcinogenic agents. The technique described may be useful in analysing analogies between human and experimental tumours.

If experiments on tumour proliferation in tissue cultures compared to tumour doubling time in vivo are performed, the exact computation of the actual tumour volume is a necessity. Another possibility are investigations of different tumour growth centres in respect to different cell types and immune responses of the host tissue. Our data show clearly that these or similar investigations cannot be performed with projective tumour data because of significant systematic error rates in the actual tumour volume.

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