THE MORPHOLOGY OF MALIGNANT TUMOUR CELLS IN SEROUS EFFUSIONS

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Pleural and ascitic fluids make up approximately 15 - 20% of the total number of specimens examined by the diagnostic cytology unit of the SAIMR. In patients suspected of harbouring a malignant tumour, the identification of cancer cells in the effusion confirms the presence of a neoplasm and indicates the prognosis. Where the cause of the effusion is obscure, the positive smear establishes the diagnosis. Although this aspect of cytology has not the significance of female genital cytology in detection and subsequent eradication of malignancy in its earliest stages, it is of value as a diagnostic procedure.

METHODS

Freshly aspirated fluid is submitted to the laboratory immediately, where five smears are prepared from the sediment of the centrifuged specimen. Two are stained with E.A. 65, O.G.6,[1] two with May-Grünwald Giemsa,[2] and one with acridine orange[3] for fluorescence microscopy.

If a coagulum is present it is fixed in Bouin's fixative embedded in paraffin, sectioned and stained with haematoxylin and eosin.

MORPHOLOGY

Metastatic tumours of the serous membranes account for the majority of malignant effusions. Primary malignant tumours, the diffuse mesotheliomas, occur far less frequently. From either source cancer cells exfoliate into the accumulating fluid, which forms an excellent medium for their continued growth.

Metastatic Tumours

Adenocarcinoma

This is the commonest metastatic tumour, and two distinct patterns are recognized:

1. The malignant cells occur in aggregations or clusters as morulae, and acini (Fig. 7). The distinctive feature is the presence of large cytoplasmic vacuoles (Figs. 1 - 6) which displace the nuclei, often compressing them, forming signet-ring cells. The nuclei show chromatin irregularity and distinct nucleoli. The cytoplasm may be basophilic or eosinophilic. In the aggregations mitoses may be noted. This presentation was found in adenocarcinoma of the gastro-intestinal tract, and uterine, pulmonary and ovarian adenocarcinoma. In mammary carcinoma the cells are less frequently vacuolated and more often occur as clusters and sheets of medium-sized and small undifferentiated cells. Fronds of malignant tissue desquamated from the tumour occasionally occur in ovarian papillary adenocarcinoma specimens.

2. Discrete isolated malignant cells (Figs. 8 - 10) constitute the chief cellular component. In size they are similar to the mesothelial cell or somewhat larger. The cell membrane is clearly outlined, the large nucleus is central or eccentric, the chromatin irregularity is variable and there is a prominent nucleolus. The cytoplasm is basophilic or eosinophilic with the Papanicolaou staining. It may contain fine vacuoles, a single vacuole compressing the nucleus, or it may be homogeneous. This pattern was noted in adenocarcinoma of pulmonary, colonic and ovarian origin, but far less frequently. Distinguishing these cells from active mesothelial cells may prove difficult.

Squamous Carcinoma

Since this tumour metastasizes far less frequently to serous membranes, the occurrence of cancer cells in associated effusions is less common.

The large cells have dense hyperchromatic nuclei with eosinophilic cytoplasm (Papanicolaou method); the eosinophilia may be intense. The nuclei exhibit pleomorphism. The cells occur singly (Fig. 11), in groups (Figs. 12 and 13), in large and small sheets (Figs. 14 and 15) and occasionally in pearl formation (Fig. 16).

Malignant Lymphoma

The striking feature is the marked cellularity of the smear (Figs. 17 - 19), owing to the excessive number of small cells forming tight groups in some areas and spread evenly in others. The small lymphocytes have scanty basophilic cytoplasm and deeply staining nuclei. The immature forms are large, the nucleus occupies almost the entire cell and there is a faintly basophilic cytoplasmic rim. Many cells are distinctly outlined, but in others cell margins are not evident.

In a malignant lymphoma of the stem-cell type, the entire population consists of primitive fragile cells with large nuclei, some of which display prominent nucleoli (Fig. 20) and a rim of faintly staining basophilic cytoplasm. Mitoses are numerous.

Reticulum-cell Sarcoma

The number of reticulum cells varies greatly. Usually they are discrete and single (Fig. 21), but small groups may occur. Mature and primitive forms are present. In the mature form (Figs. 22 and 23) the cells have distinct outlines, the nuclei are large and contain definite nucleoli, and the cytoplasm has a delicate basophilia. The primitive cell (Figs. 24 and 25) has scanty cytoplasm, the nucleus is round or irregular and the nucleoli are large and prominent. The chromatin network is that of an immature cell.

Undifferentiated Malignant Cells

Anaplastic malignant cells occur as single elements, clusters and sheets (Fig. 26). The principal feature is the nuclear pleomorphism. Mitoses may be plentiful, reflecting the active proliferation. Since cell differentiation is absent, identifying criteria cannot be applied. Therefore attempts to classify the cell type are virtually useless.

Primary Tumours

Malignant Mesothelioma

The morphology of cells derived from malignant mesothelioma resembles that of benign mesothelial cells, but the malignant cells have abnormal nuclei with prominent nucleoli. The cells occur singly as well as in groups. They vary in size and many are multinucleated. In a recent specimen (Figs. 27 - 29) aggregations of granular material were noted in the cytoplasm. This was particularly apparent in the Giemsa preparations. The possibility of this being hyaluronic acid could not be ascertained, since special techniques were not performed.

DISCUSSION

The identification of unequivocal malignant cells in effusions is reported as a positive smear. Distinctive features exhibited by the cells, and the patterns of arrangement, permit classification of the preparations into the recognizable cell types, e.g. adenocarcinoma or lymphoma. However, correlation with the site of the primary tumour,

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Figs. 1 - 3. Differentiated adenocarcinoma. Clusters of large vacuolated cells (Papanicolaou, Giemsa, acridine orange).

Figs. 4 - 6. Differentiated adenocarcinoma. Clusters of small cells, some showing cytoplasmic vacuoles (Papanicolaou, Giemsa, acridine orange).

Fig. 7. Adenocarcinoma. Acini consisting of small malignant cells (haematoxylin and eosin).

Figs. 8 - 10. Discrete, single-cell adenocarcinoma (Papanicolaou, Giemsa, acridine orange).

Figs. 11 - 16. Squamous carcinoma (Papanicolaou, Giemsa, haematoxylin and eosin).

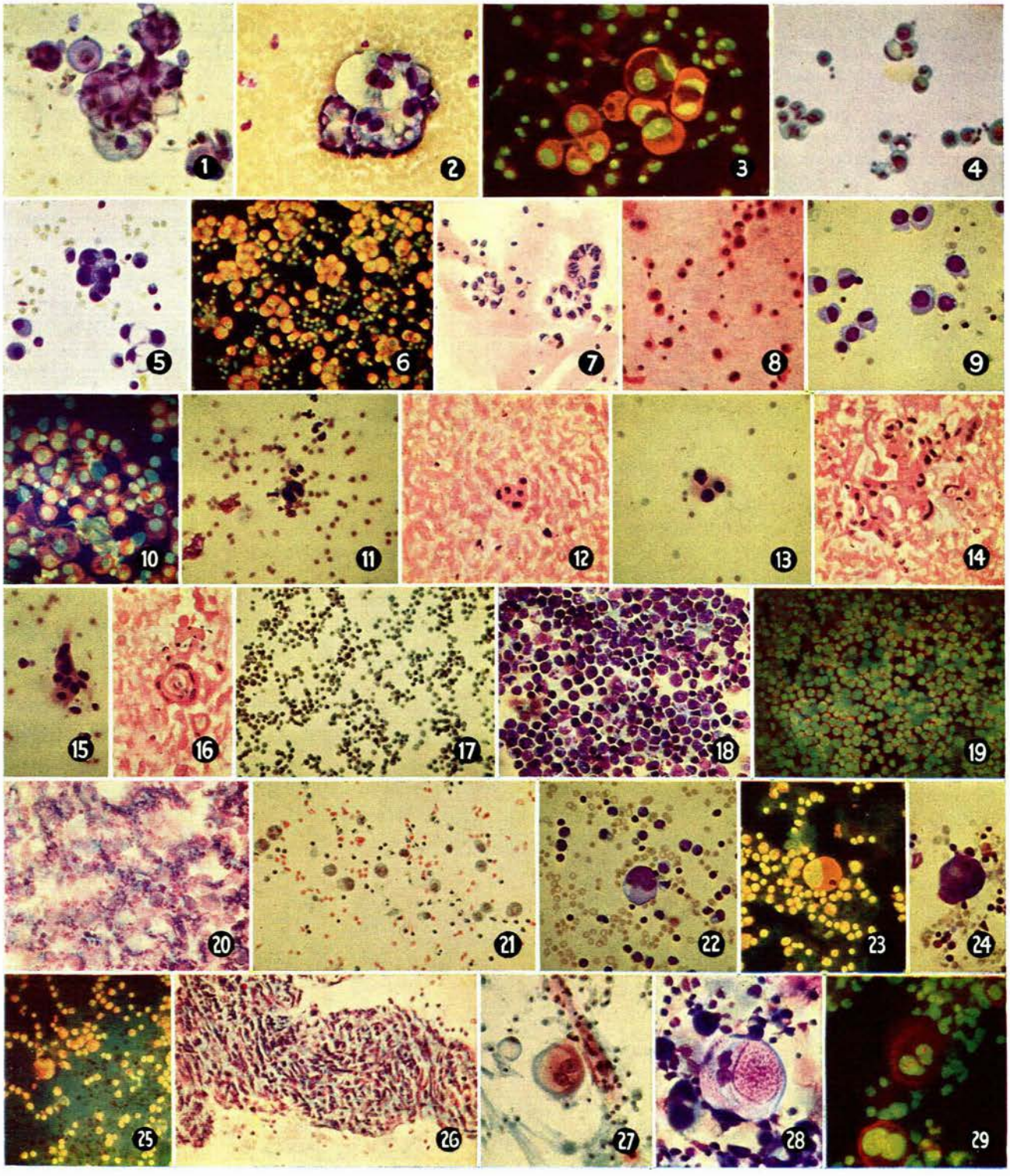
Figs. 17 - 19. Malignant lymphoma. Numerous small immature cells of the lymphocyte series. Histology showed lymphosarcoma (Papanicolaou, Giemsa, acridine orange).

Fig. 20. Stem-cell lymphoma. Note the mitotic figure (Giemsa).

Figs. 21 - 25. Reticulum-cell sarcoma (Papanicolaou, Giemsa, acridine orange).

Fig. 26. Sheet of undifferentiated small cells (haematoxylin and eosin).

Figs. 27 - 29. Differentiated malignant mesothelioma cells (Papanicolaou, Giemsa, acridine orange).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i.e. ovary, stomach or breast, is invariably less accurate. Tentative suggestions may be proposed, but exact determination is not always possible. This finding conforms with the difficulty of interpretation of histological sections of some metastatic tumours. Similarly, undifferentiated malignant cells which may be derived from sarcoma, less common varieties of carcinoma, and other miscellaneous tumours, present as anaplastic malignant tumours in histological section. Again it may not be possible to arrive at a definite conclusion, in spite of the use of special techniques.

The negative smear does not necessarily exclude malignancy. An effusion associated with a malignant tumour may occur in the absence of serosal metastases. Fibrin covering the tumour may prevent the exfoliation of cells into the fluid, or the tumour may not shed malignant cells. These factors, or the inability to recognize the cells as malignant, account for the 'false' negative result. In suspected cases further specimens should be examined.

The problem smears have atypical cells on which a definite opinion cannot be expressed. Proliferating mesothelial cells with active nuclei and increased cytoplasmic RNA synthesis, which occur in congestive cardiac failure, pulmonary infarction, virus pleurisy and cirrhosis of the liver, may simulate malignant cells. The cytological picture of malignant lymphoma may be difficult to differentiate from a tuberculous effusion in which there are many immature lymphocytes. These conditions are responsible for false-positive reports. In conjunction with all the available data further specimens should be examined in an attempt to provide a final diagnosis.

As regards staining techniques, the cytologist relies on the particular method with which he has had most experience. Papanicolaou preparations provide excellent nuclear detail upon which the diagnosis of malignancy is primarily based. In acridine orange and Giemsa techniques there is a greater concentration of cells in the smears. Immature cells in the malignant lymphomas and reticulum-cell sarcomas are more easily typed with Giemsa stains. Fluorescence microscopy reduces the screening time, particularly in the negative smear. It has proved helpful in distinguishing malignant cells from atypical mesothelial cells in some cases. Both the morphology and staining characteristics must be considered in assessing the cells. In obvious smears each technique provides confirmatory evidence. In the problem smear the various methods may contribute additional information and facilitate diagnosis. Degenerate material is generally unsatisfactory.

SUMMARY

Exfoliative cytology applied to serous effusions is of definite value as a diagnostic adjunct. Preparations from the specimens are stained with Papanicolaou, May-Grünwald Giemsa, and acridine orange stains. Distinctive features permit classification of the preparations into recognizable cell types. Correlation with the site of the primary tumour is less exact. Active and immature cells in chronic inflammatory and proliferative conditions may be confused with malignant cells. The various staining techniques confirm and facilitate diagnosis.

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