DIAGNOSTIC CYTOLOGY OF EFFUSION FLUIDS

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Cytological diagnosis is the science dealing with the examination of cells from the human body and their interpretation for diagnostic purposes. The cells may have been spontaneously exfoliated or removed by articficial means. The subject has assumed great importance in the last few decades, and the possibilities of this field of pathology seem to be by no means exhausted. The concept has been greatly influenced by the well known work of Papanicolaou on the cytological aspects of cancer in the female genital tract. Papanicolaou's methods in fact have proved instrumental in giving cytology the definite status of an approved laboratory procedure. Cytology has in fact ceased to be an adjunct to other methods of diagnosis, but has become a primary and important source of information in many fields of medicine. The competent cytologist should not only be able to suggest, but should be prepared to establish a diagnosis on the evidence at his disposal in much the same way as the morbid anatomist does on biological evidence.

This note is concerned with the cytological diagnosis of effusion fluids, i.e. "puncture fluids" from pleural, peritoneal, and pericardial body cavities. The anatomical and physiological properties of these are largely identical, with their parietal and visceral layers forming a virtual sack around the respective enclosed viscera, and containing a small quantity of fluid to lubricate the movements of the enclosed organs. Histologically, also, they are all lined by a single layer of flat cells, the mesothelium, supported by connective tissue and provided with appropriate vascular and nervous apparata.

History. Cytological examination of fluids is not in itself a novel procedure. Reports, however sporadic, appear in the literature of the late nineteenth century. These were mainly concerned with the search for malignant cells. Even cytodiagnosis as we understand it here, not limited, namely, to the investigation regarding possible malignancy, dates from the first decade of the present century. Its introduction appears to be due to Widal who in 1900, classified the differential cell-pictures of fluids into various diagnostic categories. Numerous workers in different countries followed suite, with resulting rapidly increasing numbers of publications on the subject. Lack of completely satisfactory results and the continuous search for suitable techniques of preparation and staining methods are apparent from these reports; a state of things which resulted in the introduction of the "cell-block" technique. This consists, of course, in making sections, after fixing, of the centrifuged deposit of the fluid. But again the results, if they could satisfy the histopathologist, were not what the cytologist was after. The former bases his diagnosis on cell-aggregates, the cytologist on cell-appearances. The two fields, though analogous, are not identical. The method, however, gained ground and became the standard one especially in America, until it was challenged by Papanicolaou's method. The latter has finally provided the cytologist not simply with the choice method but with the standard one. The next evolu-

tionary step, was the application of the Romanowski stains to specially prepared films from effusion fluids. The secret of the method lies in the latter technique: very thin films, that dry up almost immediately on spreading, are required (Spriggs, 1957). The method is the one we have been using for some years, with very satisfactory results.

The cells of Effusions

Under normal conditions the potential body cavities described contain a very small amount of lubricating fluid, so small that it cannot be tapped. Allegedly successful experimental tappings were reported as yielding a fluid of very low protein concentration and containing a few cells mainly monocytes, together with a few mesothelials and lymphocytes, in the region of 1500-2000 per c.mm. An amount of fluid that can be aspirated with more or less ease is in itself indicative of a pathological process, which is readily reflected in the altered cytology of the fluid. The presence of the effusion is in fact attended by the following changes.

(a) The mesothelial lining of the serous layers, now mechanically separated by the effused fluid, undergoes a slight hypertrophy, the mesothelial cells becoming rounded or cuboidal in shape and are ultimately shed into the fluid to be replaced by younger ones. This is what happens in the presence of uncomplicated transudates, the mechanically produced effusions accompanying the accumulation of fluid in the rest of the body tissues. (in cardiac, or, more often in cardio-renal disease; in liver cirrhosis; in hypoproteinaemia etc).

(b) The presence of an inflammatory process of any nature, whether primary or supervening on a transudate, brings about a greater disturbance of the mesothelial lining, which becomes greatly thickened and several layers deep. Desquamation of the cuboidal mesothelial

cells and their multiplication in the effused fluid goes on and is more pronounced; but the picture here is changed through the presence of the cells characteristic of the underlying inflammatory condition. Unlike transudates, this is an active, *exudative* process, probably associated with damaged capillary endothelium. *All the circulating blood cells,* apart from accidental contamination, may be present in an exudate, including the erythrocytes themselves, which, there is reason to believe, are produced locally. It may here be noted that pure, uncomplicated transudates are rare, and when confronted with the least complicated cytological picture we are always dealing with an exudate of some sort.

(c) If, as a result of the inflammatory process the free mesothelial surfaces become covered with fibrinous exudate (purulent pleurisy, tuberculous pleurisy, fibrocaseous tuberculosis), the mesothelial cells can no longer be shed, and they are consequently absent from the fluid. This may prove an important differential diagnostic point.

Cell pictures and their interpretation

From what has been said above, it is clear that the *cytological set up* of an effusion fluid may be quite a complex affair; and elsewhere we have said that the cytologist bases his diagnosis on *individual cell appearances*. This latter statement may sound ill-equipment on the part of the cytologist when dealing with complex pictures. It is true that in some cases, notably in malignant effusions, that all important concern of the cytologist is the detection of a particular cell, in that case the malignant cell. But it is equally true that the task of the cytologist is not merely that of accurately naming each one of the cells in the material under examination, however exacting that task may some-

times prove to be. His familiarity with cell morphology and its variations must go hand in hand with that of the significance of cell groupings and their incidence, their mutual association or exclusion. In other words he must be familiar with *differential cell-picture and their significance.* For this reason a classification of the cytological findings in effusion fluids based on cell-pictures is more profitable than one based on underlying pathological states. We shall therefore consider the interpretation of the more common and significant conditions under the following headings:—
    Mesothelial predominance.
    Lymphocytic predominance.
    Predominant neutrophil polymorphs.
    Pleomorphic cell-picture.
    Easinophilic pictures.
    Presence of L.E. Cells.

Mesothelial predominance

This is characteristic of simple, uncomplicated transudates, especially of the pleural cavities. These fluids usually contain very few cells, often requiring the centrifuging of considerable quantities of material to bring them out. Large numbers of mesothelials probably never occur is uncomplicated transudates. When they are present one is likely to be dealing with an exudate.

Lymphocytic predominance

Lymphocytes are present in varying numbers in most effusions, including *transudates,* where they accompany, and in exceptional cases may outnumber, the mesothelials. They are found in all *serous* effusions. In some conditions to be mentioned presently they may predominate even to the exclusion of all other cells. We often refer to the picture in these cases as a "purely lymphocytic fluid". Predominantly lymphocytic pictures are seen in:—
    (a) *Tuberculous effusions.* both the primary and the condition secondary to fibro-caseous tuberculosis of the lung (They are absent in empyema and in pneumothorax effusions, which are characterised by pus cells and eosinophils respectively). A varying percentage of neutrophils may be present in addition during the first few, say ten, days after the onset.
    (b) *Some post-pneumonic effusions,* which are also purely lymphocytic, mesothelial-free fluids. Obviously the case history and the M. tuberculosis-negative culture are necessary for distinguishing such cases from specific pleurisy.
    (c) *Pulmonary infarction,* probably more common than suspected, and possibly accounting for many of the pleural effusions occuring in *congestive heart failure* which yield an identical picture (closely resembling the post-pneumonic effusion, but for the presence of mesothelials, which, especially in pulmonary infarction may be rather numerous).
    (d) *Carcinoma of the lung,* which, however, should never be invoked as the cause unless malignant cells are clearly present;
    (e) Effusions complicating *lymphatic leukaemia* and the reticuloses.

Predominant neutrophil polymorphs

This picture is characteristic of the *purulent type exudate* of acute inflammation of the serous membranes with a positive culture of pyogenic organisms (a few lymphocytes and macrophages may also be present at the onset). Some of the neutrophils may show the phagocytosed responsible microorganisms which it is their purpose to eliminate (cultures soon become negative under favourable conditions). They ultimately assume the characters of *pus cell,* becoming fragile, easily smudged during film preparation, their number of scavenging macrophages results in the appearance of a *staining background*

of cellular debris: a distinguishing feature of purulent exudate. The significance of the presence or absence of mesothelials in these exudates must be mentioned once more. In the presence of the blocking fibrinous exudate in purulent pleurisy, exfoliation of mesothelials does not take place, and consequently they are absent from the fluid. Their presence in decidedly purulent exudate consequently indicates a very recent onset of the process. It is also noteworthy that at this stage the condition is still reversible, with the possibility of the purulent being turned into a serous effusion by antibiotic treatment. Otherwise the fluid remains purulent up to the complete absorption. Conversely the presence of mesothelial in doubtful *long-standing* cases will give up the fluid as non-purulent in nature.

Pleomorphic cell-pictures

These are given by the *non-purulent* type, culturally sterile exudates of acute inflammation (cf. preceding paragraph), with the presence of neutrophils, lymphocytes, macrophages, mesothelials, eosinophils and basophils, roughly in that order of frequency. They are met with chiefly in *penumonia, influenza, pulmonary collapse*. The neutrophils are here well preserved; they are not "pus cells". There is no staining background debris. The macrophages assume various, sometimes arresting shapes, mainly an expression of their accentuated phagocytic activity. The lymphocytes may be very numerous, especially in post-pneumonic cases, making the distinction from tuberculous pleurisy important (see above).

Eosinophilic pictures

In spite of the existance of an extensive literature on *pleural* and *peritoneal eosinophilia*, not much is definitely known about the condition. There is not even unanimity about what percentage constitutes eosinophilia. We shall take it to mean over 20 per cent of all the cells present. In the pleura, the condition most commonly associated with eosinophilia is *pneumothorax*, either artificial or spontaneous, whenever it is complicated by an effusion. A noteworthy exception are those cases in which the complication takes the form of a true tuberculous pleurisy, which (together with tuberculous effusion in general) is eosinophil-free. It follows that an eosinophilic effusion complicating pneumothorax is a good prognostic sign as far as the nature of the effusion goes. Large numbers of eosinophils impart a marked turbidity to a fluid.

Pleural eosinophilia is also frequently seen in *post-pneumonic* and infarct pleurisy.

There is much uncertainty regarding peritoneal eosinophilia.

Presence of L.E. Cells

Lupus erythematosus may present with a pleural effusion, in which case L.E. cells are found. There are not many cases described. We have seen an interesting case of an old woman with a long standing pleural effusion. All investigations to establish the cause proved repeatedly negative, until we detected typical L.E. cells. The accompanying picture was a highly cellular one with numerous lymphocytes, mesothelials, plasma cells, neutrophils and eosinophils. Unfortunately, no "post-mortem" could be made.

Malignant effusions

Nothing else need be stated about this type of effusion, besides the fact that they are characterised by the all-important presence of malignant cells. The detection of the latter is here the chief task of the cytologist. Malignant effusions present themselves with other characteristics, e.g. they are in general haemorrhagic (probably due to erosion

of the mesothelial blood vessels) and very often contain large numbers of lymphocytes. But, it is to be repeated, the only criterion for labeling an effusion as malignant is the presence of undoubtedly malignant cells. No report of malignancy should be given on a fluid with characters suggestive of malignancy, if there is the slightest possibility that the condition may be other than malignant. It is here that the cyologist, if called to make a diagnosis, should have at hand all the clinical particulars of the case in order to enable him to weight his expressed opinion against all the available evidence. The criteria of cell-malignancy can be definied and tabulated; but in actual practice it is often a question of "feeling". Abnormal mesothelial cells and macrophages may mimic malignant cells in appearance, giving rise to much difficulty of diagnosis even to the most experienced.

Malignant effusions are given only by secondary (metastatic) pleural tumours. Benign and primary ones never give rise to effusion, if not very exceptionally and indirectly (mechanically).

Conclusion

Cytodiagnosis is now recognised as an approved, well established laboratory method, rapidly growing into a separate branch of clinical pathology, analogous to that of morbid anatomy. Cytodiagnosis of effusion fluids, the subject of this annotation, is an important part of this science. By a special though simple technique, air-dried preparations can be made which stain well with the Romanowski stains, and yield results which are comparable for practical purposes with those obtained by Papanicalaou's method which remains the standard one. The constantly obtained differential cell-pictures are definitely diagnostic, with few exceptions, of the underlying morbid conditions.

Reference

Spriggs, A.I. 1957. The Cytology of Effusion in the Pleural, Pericardial and Peritoneal Cavities. William Heinmann: London.