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Cytologic diagnosis of carcinoma of the lung

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THE CYTOLOGIC DIAGNOSIS OF CARCINOMA OF THE LUNG

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PREFACE

In the struggle to control cancer, knowledge of the many phases of the disease process as well as the ability to recognize it in its early stages has been so inadequate that the development of any new method of approach to the problem is of importance. Any contribution to a knowledge of the disease which will permit the diagnosis and treatment of the very early lesions excites interest and comment.

Exfoliative cytology is based on the rationale that neoplastic tissue desquamates cells more readily than normal tissue and that there are certain recognizable morphologic changes attributable to these cells that will be recognized by an experienced cytologist.

TABLE OF CONTENTS

The death rate from carcinoma of the lung has remained appallingly high. The two most important factors responsible for the decrease in curability are the tardiness on the part of the patient in consulting a physician and the delay on the part of the physician in arriving at a correct diagnosis. The former can be reduced by effective education of the public and the latter reduced by increasing acuity of already well established diagnostic procedures or devising new methods of earlier diagnosis. The use of bronchial smears or smears of the sputum has made possible the diagnosis of tumor beyond the range of vision of the bronchoscope, including neoplasms situated in the upper lobes, peripherally or beyond stem bronchi and their major subdivisions.

According to Craver and Binkley (1), the first reported diagnosis of carcinoma of the lung by microscopic examination of tissue obtained by aspiration was made by Menetrier in 1886. In 1887, Hampeln diagnosed a case of cancer of the lung by examination of unstained smears of fresh sputum. In 1895, Betschart described 4 cases of malignant disease of the lung in which pieces of tumor were expectorated. Hampeln, in 1918, published a second paper concerning 25 cases of pulmonary cancer. In 13 of these cases cancer cells were found in the sputum. Dudgeon and Patrick, by using the wet film technic, in

1927, revealed that the diagnosis could be made in nearly 75% of proved cases of bronchial carcinoma. In 1934, Sweany established a diagnosis of malignancy of the lung by sputum sections of macroscopic pieces of tissue in 2 cases. Since then many authors have added other major contributions to this field.

Several sources of the material to be used in making the smears have been advocated. Dudgeon and Wrigley (2) and Woolner and McDonald (3) utilize the sputum as the source of material for making the smears. The latter authors prefer sputum to bronchial secretions to avoid patient discomfort, as does Mathews of Montreal according to Herbut and Clerf (4).

McKay et al (5) state that the advantages of bronchial smears are that sputum may also contain cells from the mouth and pharynx that must be differentiated from cancer cells and unfortunately the patient with early and resectable bronchogenic carcinoma frequently does not have a productive cough. Many patients are unable to obtain a true specimen of sputum. Therefore, McKay et al (5), Liebow et al (6), Cross et al (7), Herbut and Clerf (8) and Richardson et al (9) seem to prefer the bronchoscopic aspirations or washings as the source of the material to be studied.

Gowar (10) utilizes both sputum and bronchoscopic

specimens. Cahan and Farr (11) have devised a tracheal aspiration technique while Gledhill and co-workers (12) have utilized a needle aspiration technique.

Dudgeon and Wrigley (2) recommend the following procedure for preparation of smears from the sputum:

1. Fresh sputum utilized to avoid autolysis and lessen the digestive action of the polymorphonuclear leukocytes
2. Blood streaked fragment or more solid portions of sputum selected for the smears
3. Wet films were fixed in Schaudinn's fluid which consists of 1 vol. abs. alcohol and 2 vols. sat. aq. sol'n. of $HgCl_2$ to which 3% glacial acetic acid must be added immediately before use
4. Leave smears in fixative for 20 minutes
5. Transfer to methylated spirit to which a few drops of tincture of iodine have been added to remove the excess $HgCl_2$
6. Wash in distilled water--slides are now ready for staining
7. Mayer's haemalum is used (1 gram of Mayer's haemalum is placed in 1000 cc. water and heated to dissolve. The following reagents are added to this solution--0.2 gm. sodium iodate, 50 gm. ammonia alum and 20 cc. glacial acetic acid)

8. Stain about 20 minutes--avoid too deep staining
9. Wash with tap water where color changes from reddish-purple to blue
10. Counterstain with a weak solution of eosin
11. Take through alcohol to xylol
12. Mount with Canada balsam and coverslip

McKay et al (5) have used the following technique. An aspirator was devised from a 60 cm. end-on bronchoscopic aspirating tube to which was attached a piece of no. 12 catheter which was plugged lightly with cotton. The detachable tip (1 1/2 inches from the end) is unscrewed and cotton is lightly packed in the lumen against a thin crossbar. Secretions are caught on the cotton plug, the tip is unscrewed and the cotton plug is pushed out carrying the secretions with it. Smears are made and immediately placed in alcohol-ether fixative. If secretion is scanty, 1-2 cc. saline are injected into the suspected bronchus and the specimen is taken as before. If the tumor is central, a straight tip is used while if one of the upper lobes is suspected the curved flexible tip is inserted directly into the suspected orifice and a specimen is taken. Several smears are made.

Richardson et al (9) collected material in the bronchoscopic trap and fixed the material in formalin. They then placed it in saturated aqueous picric acid solution

for 4 hours. Paraffin sections were then made in the usual manner. The sections were cut 6-8 microns in thickness and stained with hematoxylin, eosin and orange G.

Herbut and Clerf (8) report an additional method used by Mathews of Montreal General Hospital. He collects a 24 hr. sputum specimen, puts it in a muslin bag, immerses it in Bouin's fixative which causes it to contract greatly and cuts sections of the material. A H and E staining procedure is utilized.

Cross and co-workers (7) recommend bronchoscopic examination and if lesions are seen biopsy specimens are taken. In all cases aspirations and/or washings are obtained, regardless of other findings or procedures. The material collected is rinsed immediately with formaldehyde and centrifuged at 1,000-2,000 r. p. m. for 10-45 minutes. After a minimum of 4 hours of fixation, the supernatant fluid is carefully decanted and the remaining button is wrapped in filter paper if necessary. It is then dehydrated and embedded as any other surgical specimen. Sections are cut and stained with H and E.

The tracheal aspiration technique of Cahan and Farr (11) is described as follows:

1. Premedication consists of 120 mg. sodium phenobarbital (I. M.) 45 minutes before starting procedure

to minimize the dangers of a pontocaine reaction

2. Seat the patient; apply traction to the tongue
3. Apply pontocaine as follows:
   a. Directly into both external nares
   b. Directly at oropharynx
   c. Up into nasopharynx (with mobile nozzle tip directed 90° upward)
   d. Down into the hypopharynx (nozzle tip angled 90° downward)
   e. Down on the cords and into the subglottic area (nozzle tip angled 120° downward with patient inhaling 2-3 times without coughing or gagging)
   f. Allow 10 minutes to take effect
4. The saline moistened catheter is passed into a patent nostril
5. Patient is instructed to crane neck forward with chin up and to breathe in and out freely (keep traction on the tongue); this presents an open larynx
6. If anesthetized properly, no reflex cough is elliciated as the catheter is advanced into the trachea. This may be confirmed by (a) patient's inability to phonate in falsetto, (b) the presence of an audible, high-pitched whistle of air around and through the catheter and (c) the position of the catheter in the midline of the pharynx

7. Two cc. of saline and pontocaine are slowly dropped into the trachea. After brief coughing, advance the catheter down to the region of the carina. Attempt to enter the main stem bronchi by rotating the patient's head sharply to the contra-lateral side. Varied positions may be tried in attempting to contact the lesion with the irrigating fluid
8. Irrigate and aspirate repeatedly using 2 cc. of sol'n. each time--use a total of 10 cc.
9. The catheter is manipulated up and down in the tracheo-bronchial tree and the patient is instructed to cough
10. Discontinue suction as the catheter is removed
11. Mix specimens with equal quantity of 95% alcohol
12. The patient is not to eat or drink for 2 hours

This method is supposed to give a specimen containing fewer alien cells than sputum or bronchial washings. As the technique is perfected and the volume of specimen is increased the quality and quantity of cell content will improve.

Farber et al (13) believe that the Papanicolaou smear method of fixation and staining of wet smears has been the most satisfactory.

Liebow et al (6) have supplemented their H and E stained slides by two additional methods. One slide

was stained by Masson's technique and one with a 2% Giemsa solution buffered with Sorensen's phosphates at a pH of 6.8. However, the vast majority of interpretations were made on the slides stained with H and E.

Gledhill and co-workers (12) describe the following method for their technique of needle aspiration. The tumor is carefully localized by roentgenographic studies. Premedication consists of 10 mg. of morphine sulfate by hypodermic injection 30 minutes before the procedure is anticipated. Using local anesthesia, the aspiration needle with suction on the syringe is inserted into the tumor in several places. The suction is withdrawn and the needle is removed. Pieces of tissue recovered from the syringe or expressed from the needle by the stylet are placed on blotting paper and fixed in formalin. Smears are prepared on clean slides from the remaining material. They are fixed with alcohol and stained with H and E while the clots on the blotting paper are processed as routine surgical material, embedded in paraffin and sectioned.

Sputum normally contains a variety of cells. Most of these are squamous epithelial cells from the upper respiratory tract and mouth and alveolar cells containing a variable quantity of dust particles from the lung. It is impossible to tell the anatomical source of the

superficial epithelial cells as they show no distinguishing features. Superficial cells seen in sputum smears are very similar to those found in the vaginal smears. The cells do not appear as nicely preserved as the superficial cells of the vaginal secretions and are often in large clusters surrounded by mucus. There is less desquamation of the cells of the deeper layers of the mouth and the upper respiratory tract. These cells resemble cells of the outer layer basal of the vaginal and cervacal epithelium. The round or oval cells have a deeply staining cytoplasm and a round or oval, finely granular nucleus surrounded by a good margin of cytoplasm.

A type of benign cell found in sputum is not encountered in vaginal secretions. They are very active cells of which the source is not known. These "deep" cells are round or cuboidal, having dense acidophilic or basophilic cytoplasm and a centrally located nucleus with condensation of the chromatin and sharp nuclear borders. The cytoplasmic-nuclear ratio is fairly constant.

The majority of the cells present in the bronchial secretions are those of the columnar type which are in various stages of degeneration. The ciliated columnar epithelial cells, found singly or in groups, are elongated cells usually with a pointed base and a broad

sharply defined border. When the cells are found well-preserved they have distinct cellular borders. These cells, like the endocervical cells of the genital tract, degenerate quite rapidly.

The nucleus of a well-preserved cell has a clear nuclear border. The chromatin arrangement of the nucleus is evenly distributed throughout and when stained the nucleus appears smooth and even. The nuclei are eccentric being seen most commonly in the narrow part of the cell but sometimes in the broader part. In some cases more than one nucleus will be found in the cells. The shape of the nucleus is round or oval and constant in form but sometimes considerably varied in size. The cytoplasm is broad at one end and in the well-preserved cells the cilia will be seen. At the opposite end of the cell is an extremely narrow projection of cytoplasm which stains faintly basophilic or acidophilic.

When the cells are lined in palisade formation their identification is easy. When the cells are found piled up in a dense cluster careful examination is necessary to focus on the structure of the cells. Usually those in the outer part of the cluster can be identified.

When undergoing degeneration the cell outlines break, the cytoplasm becomes irregular and frayed and the nuclei become swollen, rounder and faintly stained.

When this happens, careful attention should be given to the chromatin structure which is not clumped but remains smooth and finely granular to distinguish these cells from malignant cells.

Cells of the intermediary layer and basal cells are uncommon but are important to recognize as such. They may be found in cases of infection where the inner layers of the linings are involved. The intermediary cells are moderate in size, round, oval or polygonal and have sharply defined outlines. The cytoplasm stains blue to green to gray and the nuclei are round or oval and evenly stained. The basal cells resemble lymphocytes but are much larger. The nuclei are round or oval, uniformly shaped, evenly stained and occupy most of the cell. The cytoplasm is scanty and stains light green. Squamous epithelial cells are not uncommon in bronchial secretions.

The most common non-epithelial cells found in smears of sputum and bronchial secretions are polymorphonuclear leukocytes. Leukocytes, lymphocytes and plasma cells differ only in staining quality from those found in the blood and this varies according to the staining technique.

Mononuclear phagocytes or histiocytes are about 10-30 microns in diameter and are easily identified by recognition of ingested material. They may also be identified by their round or oval vesicular nuclei (often more than

one present) and vacuolated cytoplasm. Carbon and hemosiderin may be distinguished in these cells. Phagocytosed material stains orange-brown, brown or black. These cells resemble the histiocytes found in vaginal secretions. Histiocytes must be present to be certain the specimen is sputum and not saliva. The presence or absence of histiocytes is an aid in determining the source of the material.

Since bleeding is not a physiologically normal process in the respiratory tract, its presence should arouse suspicion. However, bronchoscopic and catheter techniques cause bleeding. Erythrocytes, blood pigment and fibrin may be present.

In pathological conditions of the lung there may be large numbers of pus-cells, lymphocytes and eosinophils. If there is destruction of lung tissue as in a lung abscess, clumps of swollen alveolar cells may appear. Occasionally small clumps of columnar epithelial cells from the bronchial wall are found, especially after bronchoscopy. These clumps of cells are to be distinguished from malignant cells and until a fair degree of experience is obtained may give rise to diagnostic difficulties according to Gowar (10).

Malignant cells occur in clumps of varying sizes and since they stain more deeply than other portions of

the sputum they are picked out fairly easily under the low power of the microscope. Malignant cells are frequently found lying in a streak of blood. A diagnosis of malignancy cannot be made on the appearance of a solitary abnormal cell.

Herbut and Clerf (8) report that the leukocytic elements and fibrin are usually minimal or entirely absent in tumor cases. When macrophages are numerous, cancer cells are found only on occasion. The smears, in cases of malignancy, appear cleaner than do those from cases of bronchiectasis, etc. Erythrocytes are usually fairly abundant. Pavement cells, most of which take an orange stain, are often increased in secretions containing cancer cells when the tumor is of a squamous cell variety.

Squamous carcinoma cells are classified in about the same way as those found in squamous cell carcinoma of the cervix. The cells vary from well-differentiated malignant cells with distinct cellular borders and well-defined cytoplasm to the undifferentiated cells with no cellular borders and no distinct cytoplasm visible.

The undifferentiated cells have irregular chromatin networks, increased chromatic material and tend to concentrate chromatin at the nuclear border. Differentiated malignant cells show much more pleomorphism in sputum

than in vaginal smears. The most common differentiated cell is the type of cell that is characterized by active and hyperchromatic nuclei. Tadpole and fiber cells are not common. The cytoplasm of these cells is usually stained a dense pinkish-orange. Since epithelial cells may have an acidophilic cytoplasm, the cytologist must examine the cells closely to detect the more specific abnormalities of a malignant cell.

Gowar (10) adds that malignant cells are large and irregular in size and shape. The nuclei vary in size and shape, contain one or more ill-defined nucleoli and as a rule some of the nuclei are hyperchromatic. Multinuclear giant cells may be found and sometimes there is definite keratinization. Often pearl formations are seen consisting of whorls of closely packed squamous epithelial cells.

In malignant flora there is a tendency for the cells to occur singly or in small clumps. The malignant cells have to be distinguished from swollen alveolar cells which may occur in small clumps; but they have smaller, regular and evenly stained nuclei which are not hyperchromatic. Cells with double nuclei may be seen and phagocytosis of pus-cells may be observed. Squamous epithelial cells which are large, stain faintly with eosin and have small, pale and regular nuclei should not

be confused with malignant cells. Bronchial epithelium may appear in the form of a cell-clump but here again the nuclei are less deeply stained and more regular and cilia may be detected by careful focusing under high power. Deep cells from squamous epithelium are distinguished by a great increase in chromatin and they have smooth nuclear borders and a very little irregular chromatin.

Cells of adenocarcinoma of the lung seem to have no differentiating factor from adenocarcinoma cells of the endometrium. Single cells are uncommon while tightly grouped clusters of cells are most frequently found. Variation in the amount of cytoplasm and vacuolated cytoplasm are common characteristics. The undifferentiated cells with little cytoplasm, of course, show little vacuolization while other cells may have marked vacuolization. Nuclei are usually eccentric. Malignancy is characterized by features found in any malignant cells.

Undifferentiated carcinoma cells which seem to be the only cells desquamated in this type of cancer show no similarity to columnar or squamous epithelium. These cells fulfill the criteria of undifferentiated cells as they have no distinct cellular borders and only a faint background of cytoplasm is present. The nuclei which vary in shape and size contain a nuclear disarrangement

common to all malignant cells. The cells have a coarsely granular hyperchromatic appearance with chromatin condensed at the periphery of the nuclei. Wrinkling of the nuclear surface and irregularity of the border are often noted. Gowar (10) states that in the majority of smears of this type the bronchoscopic biopsy has shown the growth to be an undifferentiated squamous cell carcinoma.

The malignant cells found in oat cell carcinoma are small, non-hyperchromatic and of unknown origin. The "oat cell" refers to the shape of the nuclei which are long and oval and vary in size while varying little in shape. Identification is based on nuclear structure since there is little or no cytoplasm. Nuclei are smooth and glazed in appearance; frequently, the chromatin is condensed at the periphery and chromatin structure is usually irregular. Cells are found in sheets rather than in the tight clusters of adenocarcinoma. The cells are easily distinguished from lymphocytes which are smaller, regular, less basophilic and do not occur in clumps.

Farber and co-workers (13) offer an original thought that the discrepancy in maturation of basal cells when admixed with cornified cells is a criteria of carcinoma in situ. However, it is not specific for early carcinoma and may be seen in any stage of squamous cell carcinoma of the lung.

Umiker and Storey (14) report the first case of bronchogenic carcinoma in situ that was correctly diagnosed preoperatively by biopsy together with cytologic examination of the bronchial aspirate and treated by resection. The carcinoma was found arising in an area of squamous metaplasia.

This case was similar to a case reported by Papanicolaou and Koprowska (15) in which a carcinoma in situ of the lung was diagnosed by cytological examination of sputum and bronchial washings. The bronchoscopic and roentgenological examinations were negative and the diagnosis was confirmed at autopsy after a painstaking search of the bronchial tree.

Many criteria used in the diagnosis of cancer are not always specific. Thickening and irregularity of the nuclear membrane may also be seen in benign pathological conditions. Cellular hypertrophy is often seen in dry smears but the presence of tripolar mitotic figures should not be disregarded.

In cases of metastatic disease of the lung the desquamated cells will resemble those of their origin. Metastasis to the respiratory tract is a common occurrence with many primary tumors.

Gaensler et al (16) have reported on the cytologic changes in bronchogenic carcinoma following treatment

with nitrogen mustard. It was found that nitrogen mustard produced cytological changes generally similar to those found after exposure to ionizing radiation. In the well differentiated tumors the changes consisted chiefly in (a) giant cell formation, (b) nuclear fragmentation, (c) increase in number of mitosis and (d) production of atypical mitoses. In undifferentiated tumors the changes consisted of (a) a decrease in the number of mitoses and (b) the appearance of large areas of necrosis.

Low doses of nitrogen mustard had no effect except for diminution of myelopoietic tissue in the bone marrow with deposition of hemosiderin there and in the spleen.

Toxic doses of nitrogen mustard produced (a) disappearance of lymphocytes from lymph nodes with (b) condensation of connective tissue and accumulation of plasmacytes, (c) loss of malpighian corpuscles of the spleen with deposition of hemosiderin and slight increase in the number of plasmacytes in the red pulp, (d) the following changes in the bone marrow--complete absence of blood forming cells, increase in the number of plasmacytes, deposition of hemosiderin, (e) disappearance of lipoid substance from the adrenal cortex and (f) Sertoli cells were the only remaining cellular structures of the testis.

Appel and Bronk (17) state that in their experiments on rabbits that at one time or other in the development of experimentally produced pulmonary carcinoma, exfoliated cells can be found in the bronchial secretions in 100% of the cases. In 58% of the cases, tumor cells could be found in the bronchial secretions before the tumor was grossly recognizable and at a stage at which neither roentgenologic examination nor bronchoscopy could have identified the growth.

Herbut and Clerf (8) report 73% accuracy in the diagnosis of lung cancer by the use of bronchial smears. This accuracy was attained in a series of 30 patients with proven cancer. In the same group a histologic diagnosis of carcinoma was made from tissue removed endoscopically in only 36%. Cancer cells were present in secretions from 7 cases in which bronchoscopy was negative. Sputums were examined from 5 cases in which cancer cells were present in bronchial secretions and in only one of these was found neoplastic cells.

Woolner and McDonald (3), in attempting to ascertain which source of cells offered the highest percentage of accuracy in diagnosis, report that 80% of positive specimens were sputum in the cytological examination of sputum and bronchial secretions. This was in a series of 6000 patients of which the smears from 400 patients were

diagnosed as containing cancer cells. In these patients with a positive cytologic diagnosis, 144 were explored, 55% of whom had a positive bronchoscopic biopsy and 77 cancers were resected.

Richardson et al (9) report 36 cases of pulmonary neoplasms that were correctly diagnosed from a series of 167 cases. In 4 patients the biopsy was negative but the bronchial secretions were positive and in no case was the slide negative when the biopsy was neoplastic.

McKay et al (5) correctly diagnosed 74% of 54 bronchogenic carcinomas by examination of 700 smears of bronchial secretions obtained from 170 bronchoscopic examinations. Fourteen false negative and 3 false positive diagnoses were made. The smear was positive in 14 cases (26%) that were negative at bronchoscopy.

In Boyd (18), Herbut reports a series of 1000 patients in which smears were 90% accurate in 237 proved cases of carcinoma of the lung while bronchoscopic biopsy was only 36.2% accurate.

Farber and co-workers (13), in 2 1/2 years of study on 4360 specimens of sputum and bronchial secretions from 1526 patients with various thoracic symptoms, found that 40% of the reports were false negatives when only 3 specimens of sputum were examined. This error was increased to 60% false negative results when only a single

specimen of bronchial aspiration was examined. They prefer 5 specimens on 3 different occasions. However, a very small percentage of false positive results was thought to be of great significance because a positive smear was usually indicative of malignancy.

Liebow et al (6) thought that the bronchial smear method appeared to be roughly twice as sensitive as the sputum smear method by their series of cases. However, false positive reports also appeared twice as great.

Gowar (10), in a total of 93 cases of which 65 were confirmed as probable primary growths of the lung, states that 64% had a positive sputum for malignant cells.

Cahan and Farr (11), utilizing their tracheal aspiration technique, diagnosed 11 cases in 2 of which tracheal aspirations were the sole determining factor. Two false negatives due to scanty specimens and faulty technique but no false positive reports were noted.

Gledhill et al (12) report 78.5% accuracy in diagnosis in a series of 56 patients with their needle aspiration technique. Bronchoscopy, performed 102 times on 87 patients enabled a positive diagnosis by biopsy in only 41.3% of the patients.

Anatomically the peripheral lesions had the poorest chance of a positive cytological diagnosis. Histologically the squamous and oat cell carcinoma (because of

their tendency to invade large bronchi) were most frequently positive while adenocarcinoma was infrequently so. The cytological diagnosis in extremely rare alveolar-cell tumors should be very helpful since clinical and roentgenological diagnosis from inflammatory disease is difficult and the peripheral location is against successful bronchoscopic biopsy. Because of usually intact mucosa, bronchial adenomas and cylindromas do not yield positive cytological findings according to Woolner and McDonald (3).

SUMMARY AND CONCLUSIONS

In summary, the author has attempted to present the diagnostic criteria and a few of the difficulties encountered in the cytological diagnosis of bronchial carcinoma. The technical procedure for collection of the specimens from different sources has been presented. Different processing and staining techniques have been described. Cytologic procedures preserve the individual cells with a minimum of distortion very much as they would appear if they could be stained and viewed in their original location.

Within broad limits cancerous growth which arises from a considerable number of different cell types follows a characteristic sequence depending upon its cellular inheritance. Preinvasive phases in the life cycle of malignant neoplasms can be recognized by minute changes in the cells themselves. The definite recognizable morphologic changes attributable to neoplastic cells have been enumerated and compared with cells from normal, benign or inflammatory conditions. Several pitfalls in the diagnosis of malignancy have been anticipated and explained.

Cytologic changes following nitrogen mustard therapy were outlined and are similar to those following irradiation.

The efficacy of the cytologic method in the diagnosis of cancer of the lung was compared from author to author and with respect to different technical procedures involved. The value of this method has been verified in early or peripheral lesions where other diagnostic methods fail.

The soundness, reliability and practical value of the cytological diagnosis of carcinoma of the lung has been well established and is now more generally recognized. Its specific advantages and its superiority in some respects over other diagnostic procedures are apparent and are no longer disputed. However, this method should not be relied upon as sole diagnostic evidence but rather it is intended to supplement the ever growing armamentarium of diagnostic procedures.

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