



Opinion

How to prepare optimum cell blocks in lung cancer: Opinion

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Abstract

Fine needle aspiration with cell blocks can diagnose lung cancers in high risk patients. As ancillary study is important for lung cancer diagnosis, subtyping and prediction of targeted therapy response, it is important that cell block made have good cellularity and morphology. Here we describe the novel cell block preparation technique for pulmonary cytology specimens in which sediments from centrifuged aspirates were collected on layered filter papers followed by application of partially melted 2% agarose gel on cell button. Out of 41 cell blocks, 22 cases of lung cancers were subtyped without immunohistochemistry. Immunohistochemistry was done in 14 cases for subtyping. Four cases showed only benign respiratory epithelium with fibrosis. We found adequate cellularity in all cases (100%), however confirmatory diagnosis could not be given in 4 (9.7%) cases.

Lung biopsies though safe and well tolerated can cause bleeding, pneumothorax, pain, shortness of breath in some patients [1]. Though fine needle aspiration can diagnose lung cancers in these high-risk patients without much complications, conventional cytology cannot do subtyping of non-small cell lung carcinomas. Ancillary study on cell block is required for lung cancer diagnosis, subtyping and prediction of targeted therapy response [2]. Cell Blocks (CBs) are considered very useful diagnostic tool to carry out Immunohistochemistry (IHC) and molecular testing [3]. It is important that cell blocks made have good cellularity and morphology to avoid interpretational errors. Inadequate cellularity of CBs is a common problem due to lack of standardization of cell block preparatory methods. Inadequate cellularity of CBs during the molecular analysis of lung cancer have been reported from 6.4% to 57% [4]. We have tried many cells blocks techniques on gynaecological samples and we have observed that cell blocks made by filtration-based technique was easy, fast and cost effective. It also could handle multiple samples simultaneously. The overall loss of tissue particles during tissue processing was very minimal [5]. In lung cancer diagnosis, where biopsies were not possible, we used same technique on pulmonary cytology specimens and found it very effective.

In our practice, for lung cancer diagnosis we make cell blocks from ultrasound/computed tomography guided aspirations, EBUS (Endo Bronchial Ultrasound) guided aspiration and from

body fluids with or without Rapid on-Site Evaluation (ROSE). The radiologist or pulmonologist assesses the size, location, vascularity and needle tip path. Then he or she performs the FNA using 12-14 brisk passes with continued suction. Needle size is selected (usually 21 gauge) based on vascularity and location of the tumour. Initial one or two smears are quickly made, stained and examined by a pathologist. We use rapid Giemsa stain or 1% toluidine blue stain for ROSE. It is important to standardise and validate initial tests. If cells are not seen in microscopic examination, a repeat test is usually performed. It is a tendency to keep on repeating the procedures till the adequate material is seen on cytology slides. As per experience, only one or two ROSE procedures are sufficient. Too many procedures for ROSE are not very useful especially if standard procedure is followed and needle is in the lesion. Multiple procedures also increase the risk of complications. Best is to prepare one or two optimum slides and then collect material directly in the formalin for cell block preparation. Material in the formalin vial is assessed for the cellularity and repeat procedure is planned accordingly. Usually 3-4 repeats can collect enough material for the diagnosis and ancillary study. When adequate material present, it appears as dispersed shiny white particles, mixed with blood sometimes, both on smear and in vials.

For cell blocks, aspiration material is collected in 10% neutral buffered formalin. It is best to avoid shaking and dispersal of particles. This is a very important step as formalin fixes the



tissue by cross linking the proteins [6]. Initial vigorous shaking can break the particles, which will be difficult to retrieve later on. We usually fix the cell blocks material up to 6–8 hours. It is best to avoid fixation beyond 24 hours as over fixation can mask the antigens and loss of immune-reactivity of epitopes on immunohistochemistry [7].

After adequate fixation, material is transferred to the test tubes and centrifuged. Sediments are taken on filter paper by pipette, gently and slowly, so that all the fluid is absorbed. Drop of eosin is put to visualise cell button. We have used modified device [8], for cell block preparation, but it can also be prepared without device. Partially melted 2% agar gel (in microwave or hot air oven) is put on sediments gently encircling beyond the edge without touching the tissue particles. It should not be very hot when applied on cell button as this can produce heat artefact. That is why we used partially melted agar as it is not very hot and cools down fast. When it solidifies, it bonds all the separate tiny particles into one single particle which is wrapped in filter paper and processed as routine histopathology specimen.

Final cell block has two layers, cell button and gel on it. While embedding, the cell button faces the cutting surface of the paraffin block and gel side remains up in the paraffin. During centrifugation process, heavy particles with cancer cells sediment faster and are at the bottom of the test tube. This portion of the sediment faces the cutting surface in a paraffin block, thus excess trimming is not required to visualise the malignant cells.

For body fluids, we put formalin on sediments and re-centrifuge the samples. Immunohistochemistry on body fluids are sometimes challenging due to presence of inflammatory cells and reactive mesothelial proliferation. It is important to triage the samples for morphologically malignant cells before ordering costly multiple immunohistochemical stains.

We have observed that above method retained all the particles with preserved morphology without any crushing, drying or heat artefact. Most of the tissue material was enough for immunohistochemistry and molecular study. When cell block material directly taken on paper or foam without fixation, we observed drying and crushing artefacts. Heat artefact was common with agar gel technique. We preferred to suspend the cell block material in 10% neutral buffer formalin and avoided direct application of hot agar gel on sediment. This preserved the morphology and retained the architecture, which was similar to biopsies. Though it is a now a routine lab practice, initially we made 41 cell blocks from lung aspirations with

this technique. Out of 41 cell blocks 22 cases of lung cancers were subtyped without immunohistochemistry. IHC was done in 14 cases for subtyping. All body fluid cell blocks required ancillary study. Molecular study was performed in eight cases. Four cases showed only benign respiratory epithelium with fibrosis and repeat procedure was performed to rule out malignancy. Though cellularity was adequate in all cases (100%), confirmatory diagnosis could not be given in 4 (9.7%) cases. We found that our unsatisfactory specimen rate is lower in comparison to those reported by other authors. Harada, et al. [4] reported unsatisfactory cell blocks specimen in 25.6% cases, Dong Z, et al. [3], reported that in 11.69% cases and Kuusakoski, et al. reported 21.5% nondiagnostic cell blocks in large series [9]. Thus, described novel technique here can make cell blocks with adequate cellularity and good morphology giving conclusive diagnosis in most of the cases.

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