BIOPSIES OF PAEDIATRIC MALIGNANCIES: Tissue Requirements for Pathology

Introduction

The biopsy of a suspected paediatric malignancy is an invasive process that carries significant risks for the patient. For this reason, it is important that the biopsy procedure secures an adequate and representative sample of the tumour, and that the specimen obtained is handled properly as it transits from the patient's body to the pathology department.

How Much Tumour is Sufficient?

An adequate specimen is one which enables the pathologist to render an appropriately confident and accurate diagnosis of what the underlying malignancy is.

Some guidelines are as follows:

1. Biopsies should be of viable tumour. Necrotic tissue, blood clot or reactive tissue surrounding the tumour are of no diagnostic value and should be avoided.
2. Biopsies from different localities of a large tumour are important to determine if there is tumour heterogeneity. It should be noted that a biopsy only samples a very small proportion of the actual tumour, and representativeness of sampling is important for accuracy of diagnosis.
3. The amount of tumour required for a diagnosis varies with the type of tumour. Some tumours such as osseous and cartilaginous tumours require far larger amounts of tissue (curettings rather than trucut biopsies) for diagnosis.
4. Although the required minimum biopsy size is difficult to state, a very crude rule of thumb is that a confident diagnosis is very difficult to make in a single core of tissue less than 1 cm in length. Stated differently, it is desirable in most cases to have at least several good and thick cores of tumour, each measuring more than 1 cm in length. The pathologist appreciates that this may not be possible in some cases and will always try to make do with whatever can be obtained in such situations.

What Should I Do with the Tissue?

The key principle to be applied in answering this question is that the biopsy specimen should be kept as intact and unaltered as possible as though it were still part of the original tumour within the patient's body.

Some guidelines are as follows:

1. It is important to be as gentle as possible with the specimen. Crushing or cauterizing the specimen leads to artefacts that usually distort the appearance of the tumour. Morphological evaluation is the cornerstone of tumour diagnosis, hence artefacts add a layer of unnecessary difficulty to the diagnostic task of the pathologist. This

is important for all tumours but is particularly important in tumours such as lymphomas and brain tumours which are composed of delicate tumour cell.

2. The easiest thing to do is to transfer the specimen as quickly and gently as possible from the biopsy instrument to the side of a clean plastic container, and then screw the lid on securely thereafter.
3. Avoid using any absorbent material such as filter paper or the sterile material that forms the wrapper to sterile surgical instruments (which for some reason has become the material of choice to place biopsies on in KK Hospital). Absorbent material leaches fluid from the biopsy, leaving the pathologist with a desiccated specimen that often has fragments of paper-derived cellulose piercing the specimen when seen under the microscope. If it is necessary to place the biopsy on paper, an alternative would be some form of un-absorbent material. If for some reason it is necessary to use filter paper or something similar, an alternative would be to soak the filter paper in normal saline so that it becomes more or less isotonic to the biopsy, reducing the amount of fluid leached from the biopsy.
4. Try to send the specimen to the pathology department as quickly as possible. Ensure that the specimen is not shaken, dropped, crushed, heated, frozen, or otherwise physically traumatized. Remember that the specimen begins to degenerate through the process of autolysis the moment it is removed from the human body, and the goal is to get it to the pathologist with as little tissue degeneration as possible. It is difficult to give a cut-off time, but a crude guide would be that more than half an hour at room temperature is not ideal.

Why Can't the Specimen Be Placed in Formalin (like Adult Specimens)?

The simple answer to this is that paediatric tumours are different from adult tumours. Most adult malignancies can be diagnosed using material fixed in formalin. In contrast, many paediatric malignancies require ancillary tests for diagnosis and prognostication such as molecular testing, electron microscopy and conventional cytogenetics. Many ancillary tests cannot utilize formalin-fixed samples, so it is important not to simply place the biopsy in formalin.

What Does the Pathologist Do When He Receives the Specimen?

When the pathologist receives the specimen, he/she determines, on the basis of clinical history and what the tumour is likely to be, how much tissue should be apportioned for the various tests that may be required.

These are the different things that may be done with the biopsy:

1. Formalin-fixation for routine paraffin sections – In spite of all that has been said, formalin-fixed paraffin-embedded (FFPE) sections remain the fundamental means by which the pathologist examines the specimen. The priority is always to have sufficient tissue for FFPE sections. With paraffin sections, the pathologist can perform special histochemical stains and immunohistochemical stains which are often important in the diagnostic process. Increasingly, more and more molecular diagnostic tests can be performed on FFPE sections (e.g., FISH, RTPCR), but some compromise remains, such as in terms of sensitivity.

2. Snap frozen tissue – This involves snap-freezing tumour tissue, usually in a cryovial, in liquid nitrogen. Such tissue is the best for molecular diagnostic tests (such as RT-PCR). If consent is given for tissue harvesting, such tissue is usually also snap frozen. It should be noted that tissue for diagnosis is always accorded priority over that for research. Tissue is apportioned for research only if the pathologist is sure that there is more tissue than required for diagnosis.
3. Embedding in Optimum Cutting Temperature (OCT) medium – OCT medium is what tissue is frozen in for frozen sections. After the frozen section is performed, the tissue can either be thawed and routinely processed as FFPE sections, or it can be kept frozen, in which case it is as good as snap frozen tissue and has the advantage that the exact nature of the tissue has been examined by means of a frozen section. One thing to note is that performing a frozen section introduces marked artefacts which make it very difficult to appreciate fine and detailed morphological features both on the frozen section itself (that is why frozen section diagnoses are usually so challenging) and in the subsequent thawed FFPE section. For this reason, the pathologist will never subject the entire specimen to a frozen section because he/she wants to ensure that there is always sufficiently tissue processed the standard way for FFPE sections.
4. Electron Microscopy – Tissue for electron microscopy is fixed in glutaraldehyde rather than formalin. Only very small amounts of tissue (1 mm3) are required. Tissue that has been formalin fixed can be placed in glutaraldehyde with no significant compromise in tissue quality.
5. Conventional Cytogenetics – Tissue for this is placed in culture medium. It is important that the specimen is clean i.e., without bacterial contamination since bacterial proliferation in culture medium can ruin this test.
6. Cytological (touch or crush) preparations – Cytological specimens can sometimes be very useful for diagnosis, as in many brain and haematological malignancies. Such preparations require unfixed tissue.

When In Doubt…

It is good practice to communicate with the pathologist prior to performing the biopsy procedure.

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