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COMMENTARY

The evolving role of image-guided biopsy and specimen fixation—update from an oncology setting

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ABSTRACT

Image-guided biopsy is well established in clinical practice, however a recent shift towards “personalized medicine” and genomic research, particularly in the oncology setting, has resulted in a greater demand for tissue, not only at preliminary diagnosis but at multiple time points in the patient’s journey. Research into the molecular pathobiology underpinning cancer development and progression continues to identify diagnostic, predictive and prognostic biomarkers that help determine and guide treatment both at the outset, and as patient’s progress or recur. This extensive tissue analysis however, necessitates larger tissue cores and a greater number of biopsies with correct fixation of the specimens obtained. We discuss the impact that this shift towards genomic medicine has taken on both radiologists and histopathologists and stress the importance of correct specimen preparation as well as biopsy technique to maximize diagnostic yield, by reviewing different methods of specimen fixation that are now required in clinical practice dependent on the clinical question posed.

INTRODUCTION

Image-guided biopsy is now well established in clinical practice, due to its proven accuracy (90–95%), low complication rate (<1%) and minimally invasive approach.¹ Its role has primarily been in providing samples for histological diagnosis using haematoxylin and eosin staining, supplemented by immunohistochemistry when required. In this update to a 1992 paper by one of the authors, we will address how the increasingly sophisticated knowledge of the molecular pathobiology underlying cancer development and progression has led to a shift towards delivering tissue samples more suitable for multimodality interrogation to facilitate personalized medicine.

“Personalized medicine”

The personalized, stratified or precision medicine approach is epitomized by its tag line of “the right drug at the right time for the right patient.” This move away from the “one drug fits all” strategy is achieved by characterising disease at the molecular level to tease out mutually exclusive disease classes and subgroups requiring different therapies. The DNA, RNA or protein markers evaluated may be diagnostic, prognostic, predictive of response to certain targeted therapies or a combination of these categories. A number of predictive markers

are now analyzed as standard of care in different cancer types (Table 1).

The practice of personalized medicine relies on new generations of cancer tests that comprehensively characterize a patient’s tumour at a molecular level and identify different biomarkers before treatment is initiated, so that physicians can identify likely responders. This is not only at initial diagnosis, but also when treatment decisions need to be made as cancers progress or recur. This therefore has a direct effect on both radiologists and histopathologists, as both larger tissue cores and a greater number of biopsies are required for more extensive analysis.

Impact on workflow

At our tertiary oncology centre, a wide range of biopsies are performed for both primary and secondary malignancies. We have seen a steady increase in the number of image guided biopsies requested over the last 4 years rising from 500 in 2014/15 to 659 in 2017/18 (32% increase). Over the last year, a proportion of patients required a repeat biopsy (5%), to provide further samples for genetic analysis or research. 1% of these however, were performed due to suboptimal handling or storage of a preceding specimen.

Table 1. Selected solid tumour types with relevant predictive markers

Disease indication	Drugs	Molecular marker predictive of response
Breast cancer Metastatic gastric cancer (trastuzumab only)	Tamoxifen Selective oestrogen receptor modulators Aromatase inhibitors	Oestrogen or progesterone receptor protein expression
	Trastuzumab Trastuzumab emtansine (antibody-drug conjugate) Pertuzumab	HER2 protein overexpression or gene amplification
Metastatic colorectal carcinoma	Cetuximab	wild-type <i>KRAS</i> and <i>NRAS</i> , i.e. lack of mutation
	Panitumumab	
Non-small cell lung carcinoma	Gefitinib	EGFR mutation: treatment sensitising or evolution of treatment resistance mutations such as T790M
	Erlotinib	
	Afatinib	
	Osimertinib	
	Crizotinib	ALK or ROS1 gene rearrangement
	Ceritinib	
	Pembrolizumab	PD-L1 expression
	Nivolumab	
Head & neck squamous cell carcinoma	Nivolumab	PD-L1 expression
Gastrointestinal stromal tumours	Imatinib	KIT/PDGFR α mutation
	Sunitinib	
Malignant melanoma	Vemurafenib	BRAF codon 600 mutation, especially V600E
	Dabrafenib	
	Trametinib	
Ovarian cancer	Olaparib	Somatic or germline BRCA1/2 gene mutation

It is perhaps understandable that radiologists concentrate more on the process of image-guided biopsy acquisition than how the specimen is best handled once obtained. Autolysis and sample degradation begin as soon as it is taken. Depending on the clinical question, the tissue needs to be preserved in a suitable medium dictated by the differential diagnosis and molecular tests potentially required. It then needs to be carefully transported to the lab to avoid damage, particularly fragmentation. There are no comprehensive guidelines for the optimal preservation of biopsy samples. The rise of genomic medicine has also added an additional layer of complexity, as emerging molecular tests in both clinical practice and research settings employ new fixation methods. Now, more than ever, close liaison with the histopathologist, with regards to the best type of

tissue preservative is therefore of utmost importance, in order to maximize diagnostic yield from our specimens.

Specimen fixation

10% neutral buffered formalin remains the primary fixative of choice and is suitable in the majority of clinical situations, as it will allow morphological analysis by light microscopy, as well as immunohistochemistry to further characterize the tissue components. Over the past few years, UK clinical pathology laboratories have been in the process of obtaining accreditation to ISO15189 standard and this requires not only evidence of validation of techniques but also adherence to established processes. Formalin is a recognized mucosal irritant and pressure has been mounting for a move away from its use over the past decade following classification as a carcinogen by the International Agency for Research Against Cancer.² A number of alternative fixatives exist and have been variably trialled and adopted for use in specific situations, including the PAXgene[®] Tissue fixative and stabilization system (PreanalytiX[™], a collaboration between Qiagen, Hilden, Germany and BD Biosciences, Erembodegen, Belgium) is the most extensively studied novel fixative in the past few years^{3,4} and is currently being piloted through experimental work as part of the 100,000 genomes project.

Fresh tissue is preferred for cytogenetic applications, *e.g.* in diagnosis and prognostication of paediatric tumours and lymphoma. Fresh tissue acquisition is also being strongly promoted by Genomics England and NHS England for the whole genomic analysis currently being performed through the 100,000 genomes project, and also the larger scope of genetic analysis proposed in the cancer test directory as part of the national genomic medicine service in England due to launch in 2018. The joint publication by Genomics England, the Human Tissue Authority, Royal College of Pathologists and others of a consensus document recognising the increasing utility of fresh frozen tissue for genomic studies last year marked an important landmark of change in routine practice.

Guidance regarding sample handling, including recommended specimen fixation for common cancers can be found in cancer data sets and tissue cancer pathways issued by the Royal College of Pathologists.⁵ This guidance is compiled in Table 2 and demonstrates that 10% buffered formalin currently remains adequate in the majority of clinical situations, with fresh samples recommended first line for lymphoma and paediatric patients. Existing variation between different institutions, determined by local resources in addition to the clinical question being posed, will however ultimately dictate the type of specimen fixation adopted.

It is therefore of vital importance that the radiologist knows the clinical question to be addressed by the biopsy they perform. This may be one of primary diagnosis, diagnostic confirmation at a metastatic site, disease progression or transformation (*e.g.* low-grade to high-grade B cell lymphoma or small cell transformation of lung cancer) or the increasing requirement for extra tissue for molecular analysis for diagnosis or research purposes. It is also crucial this information be transcribed onto the pathology specimen request for the reporting pathologist, so that they can advise on the most appropriate fixation method and do not waste time or

Table 2. Specimen fixation recommendations

Disease indication	Recommended specimen fixative
Breast cancer	10% buffered formalin
Primary bone tumours	Fresh/unfixed
	10% buffered formalin
Soft tissue sarcomas	10% buffered formalin
	Fresh
	Frozen
Cancer of unknown primary	10% buffered formalin
	Fresh
Gastrointestinal and pancreatobiliary pathology	10% buffered formalin
Gynaecological pathology	10% buffered formalin
Head and neck pathology	10% buffered formalin
	Occasional fresh samples for vesiculobullous lesions
Lung	10% buffered formalin unless prior arrangements for fresh samples to be submitted
Lymph node and bone marrow	Fresh and/or fixed in formalin depending upon local arrangements and facilities
Paediatric	Fresh samples
Dermatopathology	10% buffered formalin
Urological pathology	10% buffered formalin

This table has been compiled from individual cancer data sets and tissue pathways published on the Royal College of Pathologists website⁵.

tissue, searching for information or confirming a previously established diagnosis with unnecessary immunohistochemistry.

CONCLUSION

In this era of genomic medicine, biopsy specimens have become even more critical for ensuring optimal diagnosis and treatment throughout the patient's journey, with a greater demand for tissue for histopathological and subsequent molecular analysis.

The resource implications of this resultant increase in biopsies on both radiology and histopathology need to be considered. Whilst the focus has previously been on biopsy technique, what we do with our biopsy specimens is just as important now, if not more so than it was in 1992 and demands greater collaboration between oncology, histopathology and radiology to ensure maximum diagnostic yield for optimal and fully informed patient management.

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