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Review

Principles of musculoskeletal tumors biopsy

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Abstract

Biopsy remains a cornerstone in diagnosing musculoskeletal tumors, requiring meticulous planning and execution to ensure diagnostic accuracy while minimizing patient harm. Some benign lesions and hematological diseases can be diagnosed through imaging and laboratory results, negating the need for biopsy. However, if a biopsy is indicated, it should be performed in a specialized, multidisciplinary center where radiologists, pathologists, orthopedic surgeons, and oncologists collaboratively determine the most appropriate approach.

Several biopsy techniques are available, each suited to different clinical scenarios. Fine Needle Aspiration (FNA) is minimally invasive but limited by its inability to provide comprehensive histological data. Core Needle Biopsy (CNB) offers tissue samples sufficient for histological and molecular analyses and is nowadays the first-line choice due to its high diagnostic accuracy and low complication rate. Incisional biopsy, while invasive, is reserved for cases where extensive tissue sampling is required or the CNB is unsuccessful. Imaging guidance, such as ultrasound, fluoroscopy, or CT, can improve diagnostic accuracy and safety, especially for deep or complex lesions (13). A well-planned biopsy respects oncological surgical principles, avoiding contamination of surrounding structures and preserving future treatment options. Errors in biopsy technique can delay diagnosis, impact prognosis, and reduce the feasibility of limb-sparing surgery in malignant cases. Complementing traditional methods, liquid biopsies are emerging as a transformative tool in oncology. By analyzing circulating tumor cells (CTCs), tumor DNA (ctDNA), and extracellular ves-

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icles (EVs), liquid biopsies offer real-time insights into tumor behavior, metastases, and chemoresistance. These advancements not only enhance diagnostic precision and personalized treatment but also promise to reduce healthcare costs and improve patient outcomes.
This review highlights the importance of biopsy planning and selection in the context of musculoskeletal tumors, emphasizing the need for specialized, multidisciplinary input to optimize patient outcomes.

Keywords

Biopsy; bone sarcoma; soft tissue tumor; percutaneous; incisional; liquid biopsy

Introduction

A properly performed biopsy with subsequent histopathological examination presents the final and decisive step in the diagnostic chain of musculoskeletal tumors.

In knowledge of the patient's medical record and clinical examination as well as after the careful review of all necessary results of local imaging studies, and eventually of the systemic staging, a decision must be made, if a biopsy is necessary for the further treatment. Some benign lesions, inflammatory processes or even a underlying hematological disease can be diagnosed based on the imaging study (plain X-Ray, CT or MRI) or the laboratory findings (elevated CRP, abnormalities in full blood count), so that they don't require a histological confirmation[10].

A biopsy is a basic technical procedure, but if oncosurgical criteria aren't followed, it might negatively impact the patient's outcome, especially limb salvage in malignancy. 1982 Mankin et al. reported that biopsy associated complications occur 3 to 5 times less frequently in a specialized tumor center than in a less specialized treatment unit[2]. 2012 Schaser et al. estimated that the complication rate of a biopsy was about 9%, when performed in a tumor center while about 30% in non specialized centers[10]. Traina et al. noted that since there is no single standard biopsy approach for all these conditions and personal experience and judgment may influence biopsy decisions, it must be performed optimally in a center where experienced radiologists, pathologists, orthopedic surgeons, and oncologists (multi-disciplinary team) will preoperatively discuss the planning[7].

The purpose of a biopsy is to provide an accurate diagnosis causing minimal harm to the patient and his definitive operative treatment. The lesion should be sampled in a representative way without damaging neurovascular structures, contaminating uninvolved anatomical compartments or limiting definitive surgical treatment. Furthermore the samples should arrive in proper form to the pathologist, who has to determine if the lesion is benign or malignant, if the diagnosis is specific and if a grading of the tissue is possible, so that the treatment cascade can begin[2, 10].

Planning a biopsy

Most patients with a soft tissue tumor or bone lesion present with focal pain and/or swelling. The clinician should perform a thorough history and physical examination to evaluate important medical aspects such as the dynamic of tumor growth, the duration, the presence of pain or other side effects as well as inciting events (e. g. trauma).

In soft tissue tumors the imaging diagnostic approach usually starts with a high resolution ultrasound. If the findings are unclear or the mass is greater than or equal to 3 cm further evaluation through sectional imaging should be initiated. The best radiological study to assess a soft tissue mass is an MRI with IV contrast agent (e. g. Gadolinium). In addition plain radiographs provide important information regarding possible bone infiltration or erosions and can rule out a bone tumor mimicking

a soft tissue mass such as a prominent exostosis or a phlebolith within a hemangioma[5].

In bone tumors plain radiographs in 2 planes are usually the first diagnostic step. Many lesions such as non ossifying fibroma, fibrous dysplasia or osteoid osteoma present a pathognomonic image, so that a definitive diagnosis can be provided by a plain radiograph and a biopsy can be avoided[4]. MRI with IV contrast agent is the technique of choice when the finding is unclear and will provide additional information concerning the following biopsy. CT may also be necessary for the further planning (osteosynthesis, prosthesis etc.)

Under suspicion of bone metastasis or hematological disease (e. g. myeloma, lymphoma) further imaging including thorax und abdomen CT as well as appropriate laboratory tests should be additionally performed[10].

In any case a biopsy should be delayed until clinical evaluation is performed and the results of all imaging and laboratory studies are collected and discussed in an multi-disciplinary team, which has to clarify the followings:

1. Is biopsy indicated?
2. Which part of the tumor has to be biopsied? The biopsy should be representative of tumor histology.
3. Which type of biopsy is appropriate as to supply sufficient tissue to the pathologist?

Which is the most suitable surgical approach so that representative tissue in sufficient quantity can be yielded, avoiding vital structures like neurovascular bundles and without causing any further focal tumor cell dissemination or risking the definitive surgical tumor resection. It's crucial that the biopsy approach is discussed with the team, who will perform the actual appropriate resection of the tumor, since the biopsy tract is contaminated[1], it shouldn't violate more than one anatomical compartment and has to be resected en bloc with the underlying tumor.

Is an imaging technique for guidance necessary and if so, which one is the most appropriate? Palpable soft tissue masses, especially those that are superficial, may not require additional imaging techniques or can be easily evaluated with ultrasound guidance. For bone tumors, especially in long bones, fluoroscopy is indispensable. Lesions that are deeper and more difficult to access near vital neurovascular structures (pelvis, spine, etc.) may require CT guidance[3, 6, 13].

Biopsy techniques

The selection of the appropriate biopsy technique for the clarification of a musculoskeletal lesion is not a trivial decision. It should be as small as possible but as large as needed to provide an accurate diagnosis and it surely depends on the surgeon's experience, the pathologist's familiarity with the cytologic and histopathologic appearance of the different kind of tumors and not lately on the equipment capability of the center[4, 6]. Antibiotics should be stopped for at least 48 hours, ideally two to three weeks before the biopsy so intraoperative microbiological cultures (for differential diagnosis) can be reliable[11].

Excisional biopsy

An excisional biopsy is the complete surgical removal of the lesion. Only minor (< 3 to 5 cm) and superficial soft tissue lesions can undergo excisional biopsy. Since a malignancy prior to resection cannot be excluded, the surgical oncological criteria (margins, compartment separation etc.) should be considered, so that the definitive treatment can follow without the risk of contamination[4, 10]. Any soft tissue neoplasm located deep to the fascia is highly likely a sarcoma and should be biopsied prior to excision[12]

Fine needle aspiration (FNA)

A fine, hollow needle is inserted into the lesion directly to yield the sample. The role of FNA in musculoskeletal tumors diagnostic is very limited[12]. The procedure is mostly used for cytology examination of the aspirate (FNA Cytology) but there is rarely a possibility of histological tissue examination (FNA Biopsy). FNA's tumor diagnosis accuracy is frequently criticized. The published results vary from excellent to unreliable[8]. An ultrasound guidance may be used and several passes may be needed to increase the yield. It is the least invasive biopsy method, which can be performed even without a general anesthesia and has the lowest risk of tumor

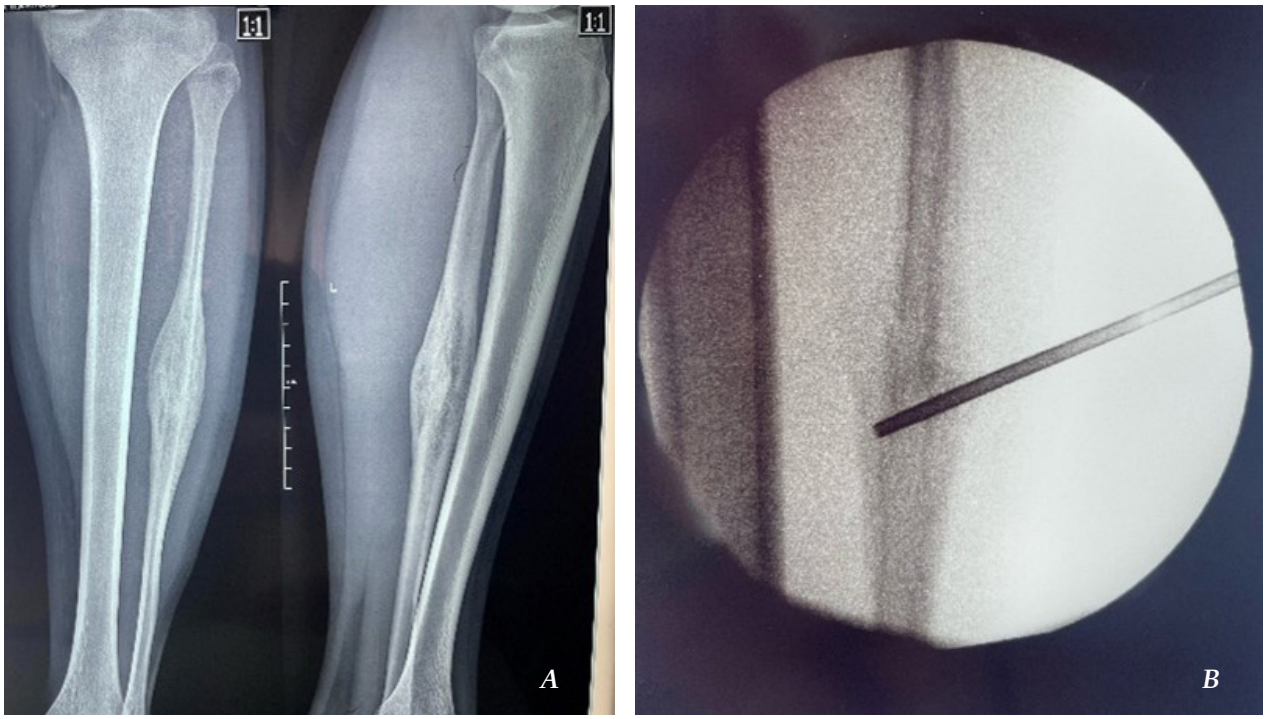

Figure 1a: *Ewing Sarcoma of fibula diaphysis links: Core Needle Biopsy (CNB) under fluoroscopy guidance.*

cell contamination of the needle tract. The primary drawback of FNA is that it only allows cytological evaluation of cells that are not structurally attached as aspirated, making it difficult to determine the histology type and grade[9].

The procedure has the highest accuracy in homogenous tumors and can be used particularly for documentation of metastases and local or distant recurrences, where the cytological findings can be compared with prior histology specimen[9, 12]. Layfield et al. reported that in 50% of all cases an FNA was followed by a Core Needle Biopsy (CNB) or incisional biopsy due to uncertain result[6].

Core needle biopsy (CNB)

Core biopsies provide a cylinder of tissue, which preserves the structural integrity of the tumor, enabling the potential for histological, immunochemical and molecular analysis and present therefore the gold standard procedure[12]. A 8-18 Gauge trocar is inserted via a small puncture wound into the mass directly or under guidance of ultrasound, fluoroscopy or CT[3, 13] (Fig 1). The site of insertion of the trocar (e. g. Jamshidi Trocar) should be in line of the possible definitive surgical incision since the biopsy tract has to be resected en bloc with the underlying tumor[1]. As this minimal tract is usually noticeable for 3-4 weeks postoperative, it should be marked (e.g. with indian ink) if the definitive surgery won't take place in the interim[4].

A minimum of three specimen for bone lesions and four for soft tissue lesions has been proposed4. Tissue samples should be taken from the periphery of the tumor due to the frequent presence of central necrosis[11]. In most cases a sufficient sample can be yielded, which can be used not only for rapid section diagnostic but also for immunohistology and molecular test such as PCR/FISH, so that an accurate diagnosis can be provided[12] (Fig 2).

CNB is usually performed as the first step in the invasive diagnostic cascade. An open biopsy is accomplished, when the sample is not representative. Especially under CT guidance CNB is very useful for deeper lesions near vital neurovascular structures, which require a pinpoint approach (pelvis, spine)[3, 9, 11, 13].

Less morbidity and fewer complications have been reported for CNB, ranking between 0-17%,

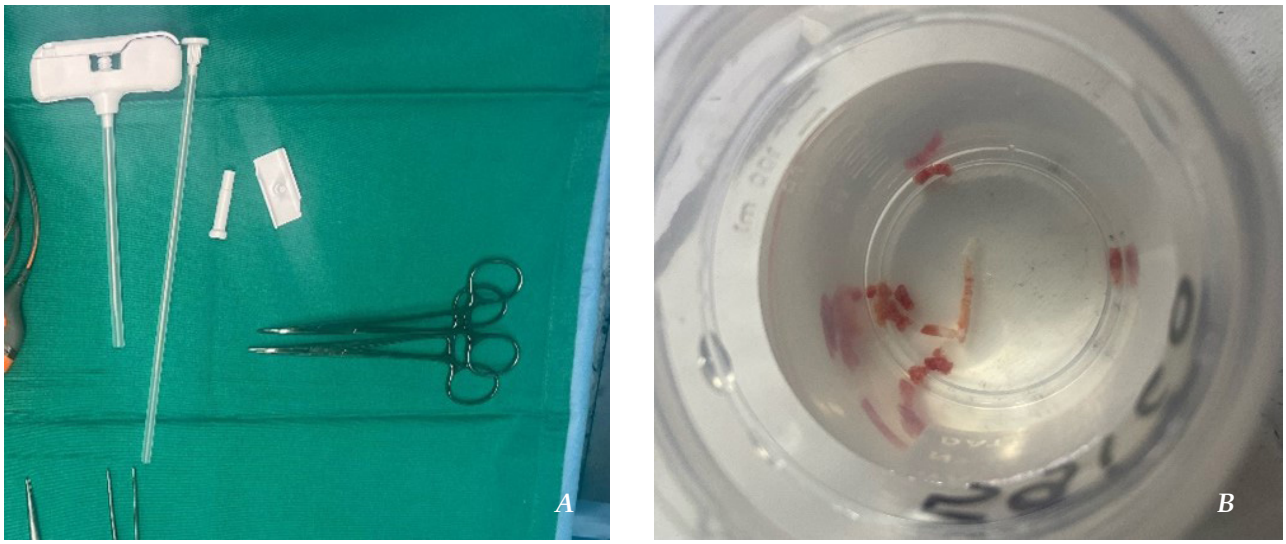

Figure 2: *a. Jamshidi Trocar is usually used for Core Needle Biopsy. b. Multiple cylinders of tissue obtained from different directions (fixation in formalin)*

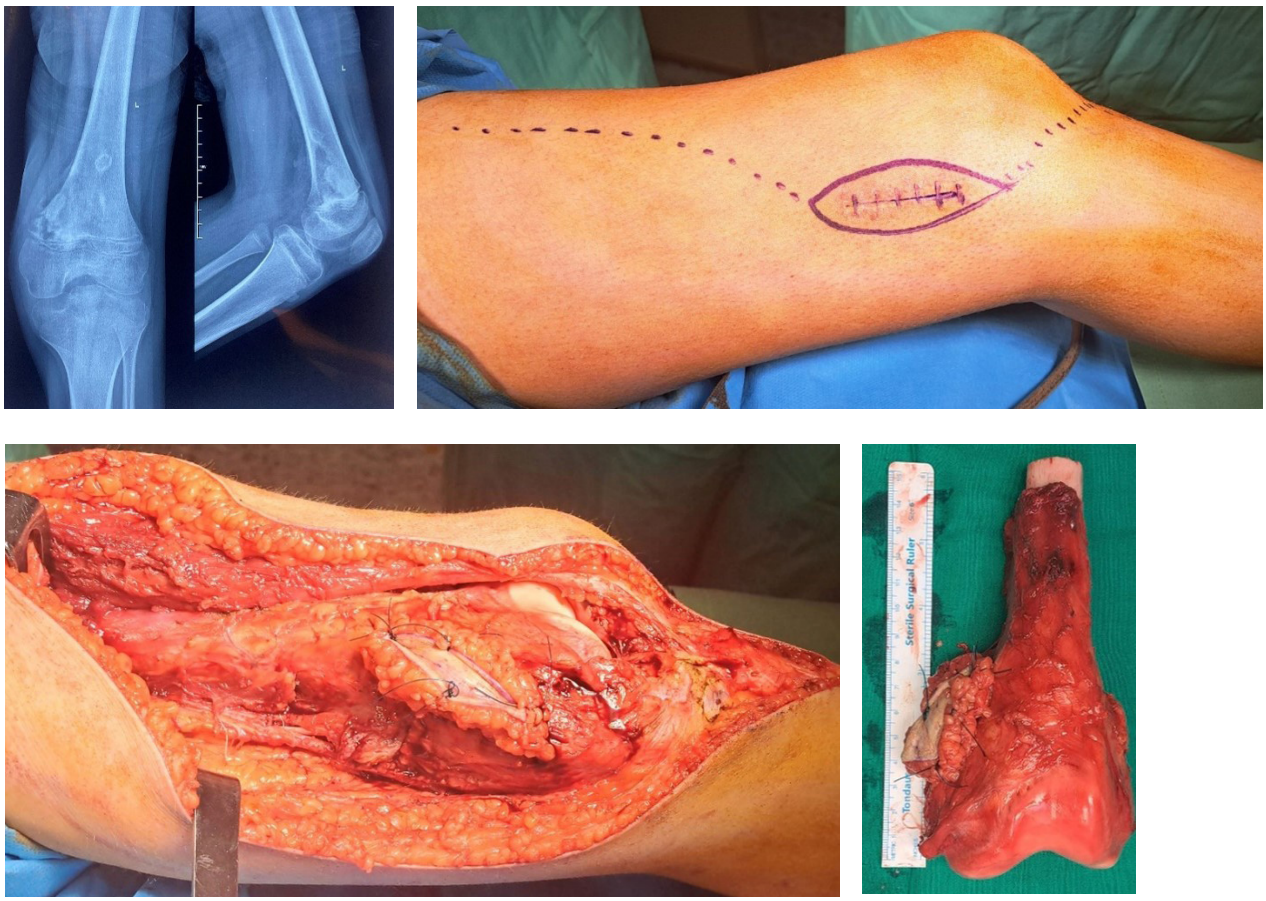

Figure 3: *Osteosarcoma of distal femur links: The definitive surgical incision (white arrow) is designed including the biopsy tract (yellow arrow), which is then resected en bloc with the underlying tumor.*

most commonly bleeding, hematoma and infection[9, 11]. Layfield et al. estimated that an incisional biopsy was performed in only 9,4% of all patients, who underwent CNB, while CNB demonstrates a slight superiority to FNA[6].

Incisional biopsy

Incisional biopsy has long been considered as the "gold standard". Certainly this is the method, which provides the largest sample of tumor tissue, allowing for higher degree of accuracy, but it also entails the greatest risk of local complications (bleeding, cell dissemination, limitation of the definitive surgery). Nowadays the incisional biopsy is indicated in difficult cases, when the imaging studies are not conclusive, the sample obtained from a less invasive method (FNA/CNB) is insufficient or larger specimen are required for further immunohistological or molecular examinations.

The sample should contain solid, vital tumor tissue of at least 1 cm^3 volume. When an incisional biopsy is performed with frozen section and the result is benign, a definitive resection can be completed in the same stage[9]. The biopsy incision must be previously discussed with the surgical team, who will perform the definitive surgery, so that the biopsy tract, which has to be completely resected en bloc with the tumor, won't influence the final surgical approach[1] (Fig. 3).

The most appropriate shortest approach (skin-lesion) should be chosen. The incision should be as small as possible and parallel to the longitudinal axis of the affected extremity. Transverse incisions are not recommended due to the need of a broader soft tissue removal during the final surgical procedure[12]. The approach should be through the muscle compartment over the tumor, no other compartment should be penetrated, so that a transfascial tumor cell contamination can be avoided. The biopsy shouldn't be in the immediate vicinity of neurovascular structures, which are vital for the extremity, or uninvolved joints. A bleeding or hematoma should be avoided too. If a tourniquet is used, a wrapping with an Esmarch bandage is contraindicated. The tourniquet should be released and precise hemostasis should be accomplished before would closure[2, 9, 12]. A drain is seldom necessary, but if needed, it should be placed closed in the line of the incision (<1 cm), since the drain sinus is regarded as contaminated and must be therefore removed en bloc with the surgical specimen and the biopsy tract[2, 9, 12].

In bone lesions with extraosseous tumor part, the soft tissue component is usually as much representative as the bony one. A penetration of the cortex can increase the risk of pathologic fracture, so that it should be performed mainly in purely intraosseous lesions[2, 7, 11]. Clark et al. reported that oblong bone windows with rounded ends provide the lowest risk of pathologic fracture. Increasing their width decreases the strength of the bone, while increasing their length does not[7].

Liquid biopsy

Liquid biopsy is a current and rapidly advancing aspect of medical diagnostics, especially in oncology. Derived from bodily fluids near malignant cells, it is particularly beneficial for tumors, that are difficult to access, as even minimally invasive biopsies can carry risks and often sample only a limited tumor area, which may not represent the entire tumor accurately.

Blood is the most commonly used fluid for liquid biopsies, though urine, cerebrospinal fluid, and saliva can also be useful depending on the tumor type. Blood-based liquid biopsies enable cancer profiling by analyzing circulating biomarkers (e.g. bone sialoprotein, osteoprotegerin), metabolites (e. g. pyridinoline) and three key biological components: circulating tumor cells (CTCs), cell-free circulating tumor DNA (ctDNA), and extracellular vesicles (EVs), such as exosomes[12].

A. Circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) are rare tumor cells found in the bloodstream, first identified nearly 150 years ago by Ashworth, and now widely utilized in cancer diagnosis, prognosis, and monitoring. Advances in technologies like FDA-approved CellSearch and next-generation sequencing enable detailed analysis of CTCs, providing insights into the tumor's genetic and molecular characteristics. CTCs are particularly significant in metastatic

cancers, with higher counts associated with worse prognosis and specific metastatic patterns in cancers like breast, lung, and prostate. For bone sarcomas, such as osteosarcoma and Ewing sarcoma, CTC levels and characteristics predict metastasis, treatment response, and disease recurrence. Emerging research highlights combined CTC and circulating tumor DNA (ctDNA) analysis as a powerful tool for assessing metastatic progression and treatment outcomes across various cancer types[12].

B. Circulating tumor DNA

Circulating tumor DNA (ctDNA), primarily derived from apoptotic and necrotic tumor cells, provides valuable insights into tumor mutations, genomic alterations, and treatment needs, such as targeting the BRAF V600E mutation in various cancers. ctDNA analysis is especially useful in detecting metastatic disease, monitoring disease progression, and identifying minimal residual disease (MRD) for prognosis and treatment planning. Studies in breast cancer and non-small cell lung cancer (NSCLC) have shown a correlation between ctDNA levels and disease progression, including bone metastases. In bone sarcomas like osteosarcoma and Ewing sarcoma, ctDNA detection via next-generation sequencing has linked genetic alterations, such as TP53 mutations and EWSR1 fusion, to tumor burden, recurrence, and poor outcomes. Additionally, ctDNA levels reflect therapeutic responses, with potential for monitoring disease recurrence and guiding treatment in sarcoma patients. Further large-scale studies are needed to refine ctDNA's clinical utility[12].

C. Extracellular vesicles (EVs)

Extracellular vesicles (EVs) are lipid bilayer particles produced by cells, including cancer cells, and play key roles in tumor biology, diagnosis, and prognosis. These vesicles, which can encapsulate DNA, RNA, and proteins, provide stable molecular information and are particularly relevant in bone metastases, osteosarcoma, and Ewing sarcoma. Research highlights the potential of EV-derived biomarkers, such as mRNA and miRNAs, for early detection of bone metastases and cancer progression, as seen in studies on breast, lung, and prostate cancers. EV cargo has also been linked to therapeutic responses and clinical outcomes, as in osteosarcoma and Ewing sarcoma, where specific miRNAs and surface proteins serve as diagnostic and prognostic indicators. Despite challenges in cost and scalability, EV-based liquid biopsies offer significant promise for non-invasive cancer monitoring and personalized treatment strategies[12].

Despite their potential, liquid biopsies face several challenges hindering widespread clinical adoption. Variations in sample collection and processing can significantly affect results, with plasma preferred over serum to avoid contamination from other DNA sources. Lifestyle factors also impact cell-free DNA release, complicating data interpretation. Circulating tumor cells (CTCs) are rare and difficult to isolate, with current methods like CellSearch limited to DNA and immunofluorescence analyses, excluding RNA-based or functional studies. CTCs may not fully represent tumor heterogeneity, though new strategies like arterial blood sampling are being explored. Extracellular vesicles (EVs) also present unique challenges, including variability in isolation techniques and difficulty distinguishing tumor-specific exosomes. Implementing liquid biopsies requires specialized training, facilities, and expertise, alongside further research to standardize protocols and enhance accuracy[12].

Conclusion

Biopsy is the ultimate step in diagnosing possible malignant and unclear musculoskeletal lesions. Technical simple but conceptually complicated, it should be extensive discussed in a specialized multidisciplinary team after the completion of clinical evaluation and imaging studies.

Due to high accuracy and low complication rate the CNB is usually the first biopsy modality, followed by an incisional biopsy, when a precise diagnosis is not possible. The use of imaging guidance (ultrasound, fluoroscopy, CT) can increase the diagnostic accuracy and reduce the risk of complications. In any case biopsy should be performed in knowledge of the definitive surgical approach, always in line with the oncosurgical principles. An improper performed biopsy can not only lead to a

delayed and false diagnosis, but it can also jeopardize the limb salvage of the extremity and affect the patient's prognosis dramatically.

Liquid biopsies, either standalone or alongside traditional methods, hold transformative potential in oncology, offering real-time tumor monitoring and detection of metastases and chemoresistance. By combining tissue and liquid biopsies, healthcare costs can be reduced while improving patient outcomes and quality of life.

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