Fine Needle Aspiration Cytology

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Introduction

Fine needle aspiration (FNA) biopsy is a technique for acquiring diagnostic material, in which a small, thin needle is used to remove cells from a lesion or mass in the body. The material that is removed consists of a sampling of the cells that make up the targeted lesion, and it can be used to prepare slides immediately for a rapid evaluation. The cells on the stained slides provide very important information about the type of process (neoplasia, infection, or inflammation) that is occurring, which is useful in explaining the etiology of the mass.

In general, cytopathology is a branch of anatomic pathology that includes aspiration or FNA cytology and exfoliative cytology (Figure 1). Exfoliative cytology involves the evaluation of cells that are spontaneously shed or manually scraped from the body, as seen in urine and body fluid specimens. However, aspiration cytology involves the use of a needle to extract cells from a lesion, and can be used to obtain a diagnosis from a variety of superficial and deep masses. The superficial sites that are commonly biopsied include lymph nodes, thyroid nodules, and masses involving the salivary gland, soft tissue, or breast. These usually involve superficial, palpable lesions that can be sampled with or without image guidance. Deep-seated masses are not palpable and, therefore, are best sampled with an image-guided FNA to help visualize the target and to guide the needle into the target. Deep lesions are usually sampled by a radiologist using ultrasound (US) or computed tomography (CT) guidance, who works closely with a cytology team, including a cytopathologist. The cytology team prepares the slides, evaluates the sample, confirms that the clinician or radiologist are in the lesion of interest, and issues a preliminary diagnosis. US-guided FNA is particularly useful for small or nonpalpable lesions, and it is most commonly used for FNAs of the thyroid, lymph nodes, soft tissue, and liver. Although these FNAs can be performed by radiologists, there are an increasing number of cytopathologists with expertise in the use of US guidance, who can also perform these FNAs. US can also be utilized with endoscopy in the evaluation of gastrointestinal, pancreaticobiliary, mediastinal, or thoracic lesions. For example, endoscopic US-guided FNA (EUS-FNA) of the pancreas and bile duct can be helpful in evaluating pancreatobiliary masses and deciding as to who needs surgical intervention. In addition, some of the more novel techniques utilizing FNA and US are the transbronchial US-guided FNA (EBUS-FNA) and transesophageal US-guided FNA for the evaluation of mediastinal lymphadenopathy and lung masses. CT-guided FNA is another way to sample deep-seated lesions and is used most often in the evaluation of lesions in the lung, bone, and kidney, as well as adrenal and other deep-seated abdominal or pelvic lesions.

Advantages and Disadvantages of FNA Cytology

FNA cytology is advantageous because it provides a minimally invasive, quick, accurate, and cost-effective way to obtain clinical answers that can guide treatment decisions. The mnemonic ‘SAFE’ is sometimes used to summarize the strengths of FNA (Tables 1 and 2). Given that FNA is a safe procedure with a low chance of complications, it can be used to rapidly alleviate patient anxiety in benign scenarios and to obtain a tissue diagnosis for lesions that do not need surgical intervention. An example is a clinical scenario of persistent lymphadenopathy that is unresponsive to antibiotics, where the clinician may decide to obtain a tissue diagnosis, as opposed to just clinical follow-up. This is advantageous, because there is a danger of possibly delaying a diagnosis and treatment for a patient with clinical observation alone, which could impact patient care. However, performing a surgical excision instead of an FNA, in a lesion with a low suspicion for malignancy, is associated with greater risks for the patient. Furthermore, FNA is beneficial for the diagnosis of diseases that are not treated with surgery, such as infections, benign conditions, and metastatic malignancy, where medical treatment is utilized. FNA may also be the only option to obtain a diagnosis for patients with deep-seated masses or those with an emergent condition (e.g., superior vena cava syndrome or spinal cord compression). Therefore, FNA provides a minimally invasive modality to get quick answers for further management.

An FNA also requires very simple, inexpensive equipment and can therefore be implemented in any physical location. Furthermore, FNA does not require anesthesia, so the procedure can be performed on an outpatient basis or at the bedside for hospitalized patients. These features also make FNA advantageous in resource-poor settings, such as developing countries or areas with limited access to hospitals or operating rooms.

FNA material can be evaluated immediately with on-site evaluation, whereby a cytopathologist evaluates the aspirated cellular material and can provide a rapid preliminary diagnosis to directly impact patient care and triage the material.
Clinicians appreciate FNA because of the fast turnaround time and feedback. Patients appreciate FNAs because they are usually not painful, and they are fast and safe. In fact, after the procedure, the patient has no scar and can resume normal activities of daily living immediately.

However, in some hospitals, FNA remains fairly underutilized, which is partially attributed to the lack of expertise available for the performance and interpretation of FNAs. In addition, there is a general misconception that some diagnoses cannot be made with an FNA needle because of the lack of large tissue fragments for the evaluation of tissue architecture. In general, the presence of trained and experienced physicians performing and interpreting FNA optimizes the performance of the FNA and decreases the possibility of nondiagnostic aspirates. This can decrease health care costs by reducing the need for additional, more costly, and more invasive procedures and can improve patient satisfaction.

The risk of complications from an FNA is low. The majority of complications that do arise occur with deep-seated targets or when larger gauge needles are used. The potential complications include a low risk of hemorrhage or hematoma, infection, pneumothorax (in deep-seated chest wall, lung, or supraclavicular FNAs), pain, vasovagal episodes, and tissue infarction or reactive changes. However, some of these risks are minimized by cleansing the area and providing pressure for hemostasis at the end of the procedure. The drawbacks of FNA include false-negative diagnoses, which are largely attributed to sampling error or suboptimal specimens, lack of architecture in some cases, and interpretation error. Sampling errors may be attributed to an inexperienced aspirator or the inherent characteristics of the lesion (e.g., fibrotic, necrotic, and cystic lesions).

Contraindications to performing an FNA include vascular carotid body tumors or pheochromocytomas, in which sampling may lead to syncope or acute hypertension. However, there are very few other contraindications. Hydatid cysts are usually not aspirated because of the risk of anaphylactic shock.
from spilled parasitic contents. In some scenarios, proximity to large blood vessels or bleeding disorders may make the procedure unsafe; however, patients being anticoagulated can have an FNA without the risk of significant bleeding, unlike other more invasive procedures.

**Comparison to Core Needle Biopsy**

A core needle biopsy (CNB) uses a hollow, larger caliber needle (usually 14–20-gauge) to remove a core of tissue from the lesion of interest. In contrast to these larger CNBs, the excursions of the FNA needle actually allow for a wider sampling area (Figure 2). In addition, the FNA sample is usually enriched in tumor cells, whereas the CNB specimen has tumor cells mixed with surrounding stroma. Aspirate slides that are generated from an FNA can be prepared quickly, have superior morphological detail, and have an entire cell nucleus on the slide, whereas histological sections of a CNB take time to fix and prepare, can have artifactual changes, and only allow a portion of the nucleus to be viewed. This nuclear truncation effect with histological sections can lead to less accurate signal counting for fluorescence in situ hybridization (FISH) tests. Furthermore, duplicate sections of a CNB and FNA cell block can easily be recut from the tissue block, in contrast to FNA aspirate smears that are unique and cannot be duplicated. CNBs also have a greater chance of complications and require local anesthesia, but they require less skill for performing than an FNA, and the sample is easier to transport (i.e., placed in formalin and sent to the lab). This is in contrast to FNA, where the smaller needle decreases the chance for complications, such as bleeding, and makes it a safer technique. CNBs can be helpful when an FNA is nondiagnostic or limited in cellularity, to obtain a fragment of tissue for evaluation. Thus, in some scenarios, these two diagnostic tools can be used in a complementary fashion to maximize the diagnostic yield. A comparison of CNB and FNA is illustrated in Figure 2 and Table 3.

**Diagnostic Performance**

In general, FNA is a very sensitive diagnostic modality, particularly when performed by those with experience in its performance and interpretation. The overall diagnostic accuracy for FNAs has been reported to be approximately 90–99%, with a sensitivity of about 85–98% and specificity of 98–100%. The accuracy is dependent on factors such as experience of the aspirator and interpreter, location of the lesion (e.g., deep lesions can be more difficult to sample), use of on-site evaluation for adequacy assessment, and the quality of the specimens. Over time, the accuracy of FNA improves with increasing experience and expertise of the cytopathologist in performing and interpreting FNAs. The use of on-site evaluation has also been shown to decrease the nondiagnostic rate of FNA cytology and improve its performance dramatically. Nondiagnostic rates tend to be higher for lesions that are difficult to sample, such as small lesions, deep-seated lesions, and some lesions with extensive fibrosis, in which it is inherently difficult to aspirate cells.

False-positive diagnoses are rare, with only occasional reports of overinterpreting scant material or overcalling cells with reactive atypia as malignant. False-negative cases are more common and are usually attributed to inadequate sampling or poor specimen quality. Failure to aspirate sufficient material, including material for ancillary studies, may also

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**Figure 2**  Comparison of fine needle aspiration (FNA) and core needle biopsy (CNB). In FNA material, cells are aspirated from multiple areas of a lesion and smeared onto a slide for cytological evaluation. In CNB specimens, a small core of tissue is removed from the lesion and then processed to create 5-μm slices of the tissue for histological examination.

**Table 3**  Comparison of fine needle aspiration biopsy and core needle biopsy

<table>
<thead>
<tr>
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<th>Fine needle aspiration biopsy</th>
<th>Core needle biopsy</th>
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<tr>
<td><strong>Sampling Specimen acquire</strong></td>
<td>Greater range of sampling Cells Tumor cells &amp; stroma</td>
<td>Limited sampling Tissue fragment Tumor cells &amp; stroma</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>Faster, immediate assessment of smear</td>
<td>Slower, wait for histological slides</td>
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<tr>
<td><strong>Size of needle</strong></td>
<td>22–27-gauge</td>
<td>14–18-gauge</td>
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<tr>
<td><strong>Local anesthetic</strong></td>
<td>Not required</td>
<td>Required</td>
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<td><strong>Advantage</strong></td>
<td>Better cellular morphology, triage of material at on-site evaluation, entire cell on slide</td>
<td>Tissue architecture, can easily get duplicate slides recut from the tissue block</td>
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<td><strong>Disadvantage</strong></td>
<td>Hard to evaluate tissue architecture, difficulty assessing for invasion, may get less material, smears are unique and cannot be replicated</td>
<td>Nuclear truncation, artifactual changes</td>
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<tr>
<td><strong>Risks</strong></td>
<td>Less, small risk of bleeding or infection</td>
<td>Greater chance of bleeding or infection due to larger size of needle</td>
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contribute to false-negative cytologic diagnoses. The success of the FNA diagnosis is really dependent on having well-prepared, optimally stained, representative material with adequate cellularity and an experienced interpreter.

**Technical Principles**

**Preprocedural Information**

When performing an FNA, there are several things to consider prior to starting the procedure, which are summarized in Table 4. Clinical history is helpful in formulating a differential diagnosis and can provide important clues to the diagnosis. For instance, a mass that persists and enlarges would be of more concern as a malignancy than a lymph node that appears to get smaller over time. Laboratory data such as laboratory testing results, serologies, and microbiology culture results can also be helpful. After obtaining a history, a directed physical examination is critical to look at the size of the lesion, mobility (mobile or fixed), contour (ill-defined or well-defined), texture (soft, doughy/cystic, or firm), and tenderness.

The FNA procedure begins by identifying a mass by physical examination or radiological imaging and determining if it is palpable. If the lesion is not palpable, then image guidance can be used to perform the FNA. The FNA itself can be performed in a variety of locations, including an outpatient clinic, a radiology suite for image-guided biopsies, an operating room, or the bedside of hospitalized patients.

The equipment required for the FNA procedure includes slides, syringes, needles (22–27-gauge), syringe holder (e.g., Cameco), sterile containers, Coplin jars with 95% alcohol, alcohol wipes, band aids, formalin containers, gauze, pencil, and personal protective equipment (masks and sterile gloves) (Figure 3). These items can be stored in a basket or cart for convenience (Figure 4). If on-site evaluation and preliminary diagnosis are desired, then a microscope and a trained pathologist are also required.

Prior to the start of the FNA, as for any medical procedure, informed consent must be obtained, including an explanation of the procedure, the benefits, and the potential complications. The consent must be signed by the physician and the patient and becomes part of the medical record. In addition, a ‘time out’ procedure needs to be performed and documented to confirm the patient’s name and identifiers, in addition to the site and side of the biopsy.

**Performing the FNA**

The FNA procedure itself involves location/palpation and immobilization, aspiration, expulsion of the material, smearing, staining, and interpretation. A diagram and animation of the FNA biopsy procedure is seen in Figure 5 and Animation 1. One of the most important factors for obtaining a diagnostic FNA is that the lesion must be localized on imaging or palpated and immobilized. The immobilization of the lesion or mass is usually done with the fingers of the nondominant hand for palpation-guided FNAs. The aspirator should have an awareness of the anatomy to avoid major blood vessels and to understand the cell types that may be contaminating the specimen from the needle pass. One example is in the case of EBUS-FNA, where the fine needle traverses the bronchial wall to sample an adjacent mediastinal lymph node. One would expect to see bronchial epithelial cells, cartilage, and mucoid material from the bronchial lumen; however, these are not the cells of interest when assessing adequacy for a lymph node FNA. In these EBUS-FNA specimens, the presence of

<table>
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<th>Table 4</th>
<th>Important preprocedural information to consider in FNA diagnoses</th>
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<td>Clinical history</td>
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<tr>
<td>Age</td>
<td></td>
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<tr>
<td>Gender</td>
<td></td>
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<tr>
<td>Location, size, and radiographic information</td>
<td></td>
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<td>Laboratory testing data</td>
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sufficient lymphocytes and/or malignant cells is required to ensure that the targeted lymph node was sampled. Thus, bronchial contamination alone would make the aspirates unsatisfactory for evaluation.

Typically, three to five needle passes are performed to ensure that the lesion is well sampled and to maximize the ability to get sufficient material for a diagnosis. The needles used are usually 22–27-gauge, disposable hypodermic needles with long bevels. If suction is to be used, then a syringe holder is helpful to be able to pull back on the syringe to use negative pressure during the FNA.

Figure 6 shows an example of an FNA syringe holder. The FNA syringe holder allows one to aspirate material using one hand. After inserting the syringe with a needle attached, the handle helps you to pull back on the syringe to use negative pressure during the FNA.

Figure 5 Technical aspects of the FNA procedure. The actual FNA procedure involves aspiration, expulsion of the material, smearing, staining, and interpretation. The multiple trajectories of the FNA needle can sample a lesion widely, and the cellular material acquired can be expelled onto a slide, smeared into a thin layer, and stained for cytopathological evaluation. Animation 1 shows this process in motion.

Attached to a needle (Figure 6). The syringe holder allows you to use one hand to aspirate; however, it increases the distance between the aspirator and the lesion, which can limit one’s fine motor control and make patients apprehensive about the size of the device. This type of FNA is advantageous in lesions that are cystic and need to be drained or when abundant material is required for ancillary studies. In FNAs are utilizing suction, negative pressure is applied once the needle is in the targeted lesion or mass and released prior to removing the needle from the target to keep the aspirated material in the needle or needle hub. Careful positioning of one’s thumb under the syringe can help stabilize the needle (Figure 7). FNA performed without suction has been referred to as the capillary method, Zajdela technique, French method, or nonaspiration aspiration. With this technique, the needle alone is used to take multiple rapid excursions through the targeted lesion or mass, and the capillary action allows the material to be drawn into the needle (Figure 8). Although there is a low yield, the material is usually highly cellular and not diluted by blood. In addition, this approach is less frightening for a patient, as it involves only the needle without the syringe and syringe holder. There is also superior fine motor control with this method, because there is a shorter distance between the hand and the lesion. This makes it the optimal technique for sampling small, mobile lesions or for sampling lesions in patients who are likely to move during the procedure (e.g., pediatric patients who are not under anesthesia). This approach is also helpful for vascular lesions (e.g., thyroid and pancreatic endocrine tumors), as there is less peripheral blood dilution when suction is not applied.

Slide Preparation

Once the material is obtained, it is expelled onto a labeled glass slide using a syringe filled with air (Figure 5 and Animation 1). The syringe helps to push the material out of the needle onto the slide. Then the material can be divided to prepare several slides and smeared to create an even monolayer of the material that will be stained for examination under the microscope. The smearing technique involves sliding a separate, clean glass slide on top of the aspirated material to make a uniform layer of material. Mastering the smear technique takes experience and is important for preparing high-quality slides, which, in turn, enhances the ability to make a definitive diagnosis.
Artifactual changes in poorly prepared smears can compromise the ability to make a diagnosis.

After smearing, the slide can be air dried or fixed in alcohol. The fixative and stain chosen will depend on the type of stain preferred by the lab and the training or experience of the individual interpreting the material. Romanowsky staining (including Diff-Quik) requires air-dried material, while Papanicolaou staining requires alcohol-fixed material. If the material is to be alcohol fixed (wet fixation), then the slide should be immediately spray fixed or placed in 95% alcohol to avoid air-drying artifact.

Papanicolaou staining is known for its superior chromatin detail and for the ability to identify squamous cells better, particularly those with keratinization (Figure 9 and Table 6). On the other hand, the Romanowsky stains are known to enhance nuclear pleomorphism and cytoplasmic detail and provide better detail of background or stromal material (Figure 9 and Table 6). In addition, Diff-Quik staining is usually preferred for rapid on-site evaluation; however, some institutions prefer to use an Ultrafast Papanicolaou stain or a rapid hematoxylin and eosin stain. The hematoxylin and eosin is a histological stain that can be used on alcohol-fixed smears and cell block sections.

### Adequacy Assessment

In general, adequacy assessment in FNA cytology is not as well defined as in Pap tests. Adequacy criteria have been developed for thyroid FNA cytology and for other tissues, there are some published guidelines indicating the minimum number and types of cells for adequacy. However, adequacy is harder to define in FNA cytology than in gynecological cytology because of the heterogeneity of the type of nongynecological aspirates that can be seen. In general, there should be a reasonable number of lesional cells from the target of interest that explain the etiology of the mass. Of the nongynecological sites, the adequacy criteria for thyroid cytology is the most well defined with a requirement of six groups of 10 follicular epithelial cells, as described in The Bethesda System for Reporting Thyroid Cytopathology.

### Table 5  Comparison of two fine needle aspiration techniques

<table>
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<th>Method of FNA</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Uses</th>
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<tr>
<td><strong>Without suction</strong>&lt;br&gt;(French, Zjadela technique, fine needle nonaspiration aspiration, capillary method)</td>
<td>• Less peripheral blood dilution&lt;br&gt;• Superior fine motor control&lt;br&gt;• Concentrated specimen with less blood (qualitative)&lt;br&gt;• Absence of syringe holder may decrease anxiety for patient</td>
<td>• Less material&lt;br&gt;• Cannot drain a cystic lesion&lt;br&gt;• Absence of syringe holder may decrease anxiety for patient</td>
<td>Vascular lesions (neuroendocrine tumors, thyroid lesions)&lt;br&gt;Small mobile lesions (e.g., lymph nodes)&lt;br&gt;Pediatric patients (not under anesthesia)</td>
</tr>
<tr>
<td><strong>With suction</strong>&lt;br&gt;(Swedish technique)</td>
<td>• Cystic lesions (to drain material)&lt;br&gt;• Can obtain more material (quantitative)</td>
<td>• Greater chance of peripheral blood dilution&lt;br&gt;• Less fine motor control&lt;br&gt;• Syringe holder may create anxiety for patient</td>
<td>Cysts&lt;br&gt;Cases requiring abundant material for ancillary studies</td>
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The goal of having well-defined adequacy criteria is to provide guidelines for cytologists evaluating FNA specimens and to minimize the possibility of a false-negative result, in addition to minimizing the nondiagnostic rate. In general, the nondiagnostic rate for FNAs should be $<5–10\%$ and has been shown to decrease significantly from 20\% to $<1\%$ with the use of on-site adequacy evaluation.

### Triage of Material

The remainder of the aspirate material that is not needed for the preparation of smears can be triaged in a variety of ways, depending on the differential diagnosis for the target of interest. This is summarized in Figure 10. For instance, if a lymphoproliferative disorder is a diagnostic consideration, then material should be allocated for flow cytometry to immunophenotype the cells, to establish clonality, and to help make a definitive diagnosis. In contrast, if infection is a consideration, a portion of the fresh aspirate can be placed in a sterile container for microbial cultures and determination of antibiotic sensitivities. Cell blocks can be prepared from aspirated material with small tissue fragments, clotted material, or residual material. The sample is centrifuged to form a pellet, which is then formalin fixed and paraffin embedded to create a cell block. The cell block sections can be used for hematoxylin and eosin staining, in addition to staining with special stains or immunostains, and for other ancillary studies (e.g., FISH studies and molecular analysis). Liquid-based cytology (LBC) is helpful for hypocellular aspirates, bloody aspirates, or cyst fluids, because it maximizes the cellular yield and removes the effect of obscuring blood. Although it was originally used for Pap tests, it has been applied to a variety of nongynecological aspirates. It is advantageous because it uses a liquid preservative solution (e.g., 20 ml of CytoLyt or CytoRich transport solution) that has an alcohol-based fixative, can be stored at room temperature, and can be given to endoscopists and radiologists performing FNAs in locations that do not have a cytopathologist or cytotechnologist to prepare smears. The aspirated material is expunged into the solution and sent to the lab, where it is processed according to the manufacturer’s instructions. Some laboratories also like LBC because the single slide produced has a clean background and is easier and faster to screen than numerous smears, which are more prone to artifactual changes, particularly if smeared by inexperienced hands. Additional LBC slides can be prepared for immunostaining or other ancillary studies.

### Ancillary Studies

There are a variety of ancillary studies that can be performed on FNA material to obtain valuable information that can help to solidify a particular diagnosis or provide prognostic or predictive information. As mentioned before, flow cytometry is helpful for suspicious lymphoproliferative disorders and microbial cultures are helpful for potential infectious cases. In addition, special stains can be utilized on FNA cytology material for the
evaluation of fungal organisms (periodic acid-Schiff (PAS) stain or Gomori methenamine silver (GMS or Grocott) stain), bacteria (e.g., Gram stain), mycobacteria (e.g., acid-fast bacilli (AFB) stain), mucin (e.g., PAS, PAS with diastase, and muci-carmine), melanin pigment (e.g., Fontana Masson stain), hemosiderin pigment (Perls or iron stain), and glycogen (e.g., PAS stain). These stains are relatively inexpensive and fast working; however, the use of immunostains has replaced the use of some of these stains. A case of a granulomatous lymphadenitis due to an atypical mycobacterial infection with a positive AFB stain is shown in Figure 11.

Immunocytochemistry involves the use of antibodies that target specific cell components and create a chromagen signal to visualize under the microscope. There are a large number of immunocytochemical stains available for the determination of cell type (e.g., cytokeratin for epithelial cells and leukocyte common antigen for lymphoid cells), evaluation and subtyping of tumors (e.g., TTF1 for lung adenocarcinomas and CDX2 for colorectal adenocarcinomas), immunoprofiling of lymphoproliferative disorders (e.g., CD3 for T-cells and CD20 for B-cells), and determination of cell proliferation (e.g., Ki67 proliferation index) and specific antibodies targeting infectious causes (e.g., viruses). An example of an FNA performed on the palate of a transplant patient, which was positive for the herpes simplex virus (HSV1&2) immunostain, is shown in Figure 12. Furthermore, immunocytochemistry can provide valuable prognostic and predictive information that can help guide treatment (e.g., estrogen receptor (ER) and Her2/neu staining in breast carcinoma). For example, an FNA case of metastatic ductal adenocarcinoma of the breast involving the liver is shown in Figure 13, with positive immunostaining for ER on the cell block.

Molecular studies, in situ hybridization, and FISH studies have also been performed on FNA cytology material with success. The material can be submitted in a nucleic acid preservative solution or extracted from the cell block or other preparations. The advantage of cytological specimens for neoplastic processes is that the material is usually enriched with neoplastic cells, with very few normal cells, and this makes the microdissection easy in comparison to histological sections where tumor cells must be removed without the surrounding normal tissue on the slide. FISH studies on FNA material are helpful in the subclassification of lymphomas and soft tissue neoplasms and can provide valuable predictive and prognostic information (e.g., FISH testing for Her2/neu in breast carcinoma). FNA smears are ideal for FISH studies because the entire nucleus is present, which allows for accurate signal counting. With all the recent advances in molecular diagnostics and immunologic methods, there are a multitude of ancillary studies available to obtain accurate and reliable diagnoses, in addition to prognostic information, and these tests can be applied to FNA cytology material when needed.
Diagnostic Approach

Overview

After preparing aspirate slides from an FNA, the slides are examined under a microscope, and the diagnostic approach to the microscopic evaluation is summarized in Figure 14 and Tables 7 and 8. The most important factors contributing to a successful FNA are having a specimen with adequate cellularity, optimal preservation and preparation, and an experienced cytopathologist. The initial examination of slides at low magnification is helpful to look at the overall cellularity, the arrangement of the cells (cohesive groups or discohesive single cells), and the background material (e.g., necrosis and inflammatory debris). The arrangement of the cells on the slide provides some architectural information about the tissue that the cells were aspirated from. Clustering may occur due to technical components (thick smear, suboptimal spreading technique, or blood clot trapping), granulomas, carcinoma,
Figure 13  Liver FNA with metastatic breast carcinoma. The aspirates show cohesive clusters of malignant cells with scant cytoplasm and occasional prominent nucleoli ((a) DQ stain, original magnification ×600; (b) Pap stain, original magnification ×600). An estrogen receptor (ER) stain performed on sections of the cell block showed strong nuclear positivity ((c) H&E stain, original magnification ×400; (d) ER stain, original magnification ×400).

Figure 14  Cytomorphological features to assess in an FNA aspirate. When examining an aspirate smear, many features are looked at to help decide the type of process that is occurring and whether the cells are benign or malignant. Some of these features are assessed at low power (e.g., overall cellularity, relationship of the cells, and background material), while other features are examined at high power (e.g., nuclear-to-cytoplasmic ratio and presence of a nucleolus).
mesothelioma, benign epithelial cells, and a variety of other processes. However, lymphoid cells, melanoma, sarcoma, and some poorly differentiated carcinoma will show more of a discohesive pattern (Table 8).

At high magnification, the focus is directed toward the type of cells present, as well as their uniformity and cell size (Table 7). In terms of lymphoid populations, aspirates concerning a malignant lymphoma tend to have monomorphic lymphoid cell populations, larger atypical cells, and absence of macrophages (except for high-grade lymphomas with a rapid turnover). Benign (so-called reactive) lymphoid processes tend to be more polymorphous, have a predominance of small lymphoid cells, and have a conspicuous presence of macrophages (Figure 15).

Cell Types

In FNA cytology, a variety of different cell types can be seen, depending on the type of lesion being aspirated and the pathological process that is occurring. Epithelial cells can be seen from aspirating normal cells from a tissue (e.g., ciliated bronchial epithelial cells in a lung aspirate; Figure 16(a)) or from an epithelial malignancy (e.g., malignant cells from an adenocarcinoma; Figure 16(d)). Epithelial cells are recognized at low power, because they typically cluster together and can be seen in sheets or other patterns. Some types of cells can also show unique cytomorphological clues that help to distinguish them from other cell types. For example, mesenchymal cells tend to appear as spindled cells with long, oval nuclei and variable amounts of cytoplasmic extensions (Figure 16(b)). Lymphoid cells can be seen in aspirates of lymph nodes, in lesions that have chronic inflammation, and in any mass involved in a lymphoproliferative disorder (Figure 17(b)). Opposed to epithelial cells, lymphoid cells and other inflammatory cells tend to be discohesive and appear as single cells, unattached from the nearby cells (Figure 17(a) and 17(b)). Lymphocytes are also recognized by the fragments of cytoplasm that can be seen in the background, which are called lymphoglandular bodies (Figure 17(b)). Other cell types include inflammatory cells, histiocytes, and multinucleated giant cells (Figure 17(b)).

Cell Arrangement

The relationship of the cells to one another is important to decide what types of cells are present, as described above; it also provides additional information. For example, cohesive clusters of cells can be seen in three-dimensional balls, two-dimensional flat sheets, or papillary structures (Figures 16 and 18). In addition, cells can be seen as single, discohesive cells (Figures 17 and 19) Crowding or overlapping and increasing complexity within clusters of epithelial cells usually raise suspicion for a neoplastic process (Figures 16(d) and 18(d)). This is in contrast to orderly, two-dimensional sheets, which are more commonly seen in benign processes (Figure 18(a) and 18(b)). In addition, certain architectural patterns, such as papillary architecture, which is usually seen as clusters of epithelial cells arranged around a fibrovascular core, can be appreciated in cytology (Figure 18(c)). Rosettes, glandular formation, or microfollicles can also be seen. Optimal slide preparation is important because discohesive cells may appear to form clusters in the presence of suboptimal technical components (thick

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<td><strong>Low-power slide review</strong></td>
<td><strong>High-power slide review</strong></td>
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<tr>
<td>Overall cellularity</td>
<td>Uniformity of cell population (e.g., homogeneous vs heterogeneous)</td>
</tr>
<tr>
<td>Cell arrangement (e.g., cohesive, discohesive)</td>
<td>Nuclear-to-cytoplasmic ratio</td>
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<tr>
<td>Background material</td>
<td>Nuclear size and chromatin pattern</td>
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<tr>
<td>**Table 8</td>
<td>Low-power pattern-based approach to making diagnoses in FNA cytology</td>
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<tr>
<td>Pattern</td>
<td>Morphology</td>
</tr>
<tr>
<td>Scant cellularity</td>
<td>Hypocellular fluid, debris, or blood with rare cells present</td>
</tr>
<tr>
<td>High cellularity</td>
<td>Numerous lesional cells seen, excluding red blood cells</td>
</tr>
<tr>
<td>Cohesive clusters</td>
<td>Cells in tight groups, sheets, or papillary groups</td>
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<tr>
<td>Discohesive cells</td>
<td>Cells that are separated from each other and do not appear attached to adjacent cells</td>
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smear, suboptimal spreading technique, or trapping within a blood clot). Discohesive cells include lymphoid cells (benign and malignant), inflammatory cells, histiocytes, sarcoma, melanoma, poorly differentiated carcinoma, and keratinizing squamous cell carcinoma (Figures 17 and 19).

**Cell Components**

Cell components often help us to decide if a cell is benign or malignant and to determine the cell type of origin (Figure 14). The nuclear-to-cytoplasmic (N:C) ratio represents the relative...
size of the nucleus and cytoplasm. Malignant cells tend to have larger nuclei that occupy a greater proportion of the cell and, therefore, an increased N:C ratio. The nucleus itself also gives us clues about whether the cell is benign or malignant. Malignant nuclei tend to be larger and have coarser chromatin, hyperchromasia, and irregular nuclear contours, whereas benign nuclei tend to be smaller with uniform, well-defined nuclear borders. The cytoplasm usually provides more clues about the cell of origin or differentiation. For example, intense orangeophilic cytoplasm on a Pap stained slide is a clue that

Figure 17 Additional cell types in FNA specimens. In addition to epithelial cells, inflammatory cells and histiocytes can also be seen, including neutrophils ((a) DQ stain, original magnification × 400), lymphocytes ((b) DQ stain, original magnification × 600), histiocytes ((c) DQ stain, original magnification × 400), and multinucleated giant cells ((d) DQ stain, original magnification × 400).

Figure 18 An overview of cellular arrangements in FNA specimens. The cell types can be arranged in a variety of different architectural patterns, including organized, cohesive sheets and clusters ((a) benign bile ductular epithelial cells, Pap stain, original magnification × 400; (b) benign mesothelial cells, DQ stain, original magnification × 600), papillae ((c) H&E stain, original magnification × 100), and crowded, complex clusters of malignant cells ((d) adenocarcinoma, DQ stain, original magnification × 400).
the cells are squamous in origin (Figure 19(b)), as opposed to vacuolated cytoplasm which is more glandular in origin. Anisonucleosis is a term that looks at the overall variation in nuclear size in the population of cells being examined. Malignant populations tend to have a greater range of nuclear size than benign processes.

Nucleoli may be variably present but tend to be more prominent in reactive or inflammatory processes and in certain malignancies (e.g., adenocarcinomas). Some malignancies are known to have distinctly prominent nucleoli, such as malignant melanoma, hepatocellular carcinoma, and Hodgkin lymphoma (i.e., Reed Sternberg cells) (Figure 19(c)). Nuclear chromatin pattern or the morphology of the DNA within the nucleus is also a key feature. In neuroendocrine tumors, the chromatin pattern is stippled or finely granular and appears as if the nucleus was sprinkled with salt and pepper (Figure 16(c)). Mitotic figures can also be seen, and an increase in number or atypical mitotic forms is usually a sign of malignancy.

**Background Material**

The aspirated noncellular background material can provide clues as to the cell types present or may represent contamination or secondary type changes (e.g., inflammation, degeneration, and necrosis) (Figure 20). The background may appear different, depending on the staining, fixation, and type of preparation. The quantitative and qualitative features of the background material can be crucial in determining how suspicious one is for malignancy. For example, in thyroid cytology, the amount of colloid is used as an important factor in determining if a nodule is benign or neoplastic. In general, the more colloid is present, the more likely the nodule is to be benign (Figure 21). Furthermore, distinctive types of background material can help in characterizing the cells present, including abundant mucin in the background of a mucinous adenocarcinoma, a tigroid background in tumors containing glycogen (i.e., seminoma), and necrosis in a high-grade malignancy or certain infections (i.e., tuberculosis) (Figure 20). Cystic backgrounds can have macrophages, including hemosiderin-laden macrophages, if there was prior bleeding, cholesterol crystals, debris, hypocellular proteinaceous material, or necrosis (Figure 20). An inflammatory background can include acute inflammation (i.e., neutrophils) or chronic inflammation (i.e., lymphocytes, plasma cells, and histiocytes) (Figure 22). A lymphoid background usually indicates a chronic inflammatory process or a lymphoproliferative disorder.

**Diagnostic Terminology**

In general, FNA cytology diagnoses involve an adequacy assessment (i.e., adequate, less than optimal, or unsatisfactory) and a general diagnostic category (Table 9). The adequacy assessment is important to convey to the ordering clinician and the performer of the biopsy that the specimen is acceptable for a diagnosis. The diagnostic categories range from non-diagnostic to negative for malignant cells, atypical cells, suspicious for neoplasm or malignant cells, or positive for neoplasm or malignant cells. In general, cases labeled atypical usually have approximately 20% chance of malignancy, whereas cases labeled suspicious have approximately 80% chance of malignancy. Although there is no universal definition about what makes an aspirate malignant, some of the features that are used in making a diagnosis of malignancy include an elevated...
nuclear-to-cytoplasmic ratio, nuclear pleomorphism and anisonucleosis, prominent or irregular nucleoli, irregular nuclear contours, and mitotic figures. In general, malignant processes tend to be highly cellular, whereas benign processes tend to yield lower cellularity or nondiagnostic specimens. Unsatisfactory aspirates include those cases with insufficient cellularity, bloody aspirates, and obscured or uninterpretable material.

A free text diagnosis and/or comment can also be added to describe the lesion, to highlight the presence of organisms, to report the results of ancillary studies, or to communicate recommendations to the clinician. As with most diagnostic tests, a negative result does not necessarily exclude the possibility of malignancy, because all FNA diagnoses require clinical and radiological correlation.

Figure 20 Overview of background material in FNA specimens. The background material in an FNA aspirate can help in understanding where the specimen came from and the type of process that is occurring. Some examples include colloid in benign thyroid nodules (a) DQ stain, original magnification ×200, cyst contents (b) DQ stain, original magnification ×400, mucinous material (c) mucinous adenocarcinoma, DQ stain, original magnification ×400, and tigroid background due to glycogen (d) seminoma, DQ stain, original magnification ×400.

Figure 21 Thyroid FNA findings in a benign colloid nodule (a) and a follicular neoplasm (b). In a benign colloid nodule of the thyroid, there is usually abundant watery colloid, which can show a cracking effect, and a few variably sized clusters of follicular epithelial cells without atypia (a) DQ stain, original magnification ×100. In an aspirate suspicious for a follicular neoplasm, there is typically scant or absent colloid and numerous follicular epithelial cells that appear in small microfollicles, as seen in this case (b) (b) DQ stain, original magnification ×200. Thus, the proportion of colloid and the overall cellularity are important factors in thyroid FNA specimens when determining if a case is benign or neoplastic.
The diagnosis then becomes part of the final pathology report that is part of the patient’s medical record and can be used to guide treatment decisions. The final report includes the date, patient identification, laboratory accessioning number, clinical history, specimen information, procedure information, intraprocedural assessment, final diagnosis, and signature of the pathologist.

**Applications to Diagnosis**

The cell samples aspirated by FNA can be used for immediate microscopic evaluation and for issuing a rapid preliminary diagnosis. A final report then follows in 24–48 h and summarizes the findings from all the slides and ancillary studies used to generate the final diagnosis. FNA cytology can be utilized to diagnose a wide variety of lesions or masses and is commonly used as a screening tool and a diagnostic tool. The primary goals of an FNA are to decide whether a lesion is benign or malignant and to further subclassify the pathologic process occurring. In the setting of thyroid cytology, FNAs are used as a screening tool to decide which patients would benefit from surgical intervention. In the setting of lymph node cytology, FNAs are used to diagnose lymphomas, metastatic carcinomas, and infectious causes of lymphadenopathy, which are not surgically treated and allow the patient to move on to clinical treatment without surgical intervention.

**Infectious Etiologies**

FNA cytology is helpful in diagnosing infection and excluding malignancy. Particularly because the appropriate treatment can be initiated quickly, patients are spared unnecessary surgery, and the material can be sent for the necessary ancillary studies (e.g., microbial cultures and special stains). This is particularly true in pediatric patients, in whom infectious and reactive lymphadenopathy outnumbers malignancy in lymph node aspirates. Furthermore, in immunocompromised patients who are at high risk of infection, cytology specimens provide an easy way to diagnose infection and initiate treatment. In the setting of lymphadenitis due to tuberculosis, FNA is a cost-effective diagnostic modality, particularly in less-developed countries with limited resources. In tuberculosis, PCR detects approximately 83% of cases, FNA detects about 50%, special staining for AFB detects about 33%, and microbial cultures detect 8–50%; thus, a combination of these modalities can optimize sensitivity and specificity. Infectious lesions can explain almost any mass lesion in the body and can be sampled by FNA. The common cytomorphological patterns seen with infection include a predominance of acute or chronic inflammatory cells, necrotic debris, granulomatous inflammation, or any combination of these findings (Figure 22). The FNA findings will depend on the time course of the infection and the organism or process responsible for the inflammatory response.

**Neoplastic Etiologies**

One of the main uses of FNA is for the diagnosis of primary or secondary malignancies. FNA is commonly used for the primary diagnosis of tumors in the lung, head, and neck, pancreaticobiliary tract, and other sites. In the lung, the use of a small FNA needle minimizes the risk of pneumothorax and provides a pathological diagnosis for a radiographic abnormality. The diagnosis will then impact the future management of
the patient. For example, adenocarcinomas and squamous cell carcinomas of the lung are usually resected, whereas small cell carcinomas of the lung are usually treated with chemotherapy (Figures 23 and 24). In some locations, the use of larger core needle biopsies has increased in the diagnosis of primary malignancies. This is particularly true for the diagnosis of primary breast tumors; however, FNA is widely used in the diagnosis of metastases, where the identification of invasion is not important. In addition, FNA is widely used in the evaluation of thyroid nodules, because the risk of malignancy associated with different diagnostic categories helps to determine who will benefit from surgery. This is important because thyroid nodules are common in adults, and the use of FNA has significantly decreased the number of patients being referred for surgery for benign thyroid nodules.

In patients with a history of malignancy, FNA can be helpful in the evaluation of metastatic disease and in cancer staging. Metastatic carcinomas are frequently diagnosed in the lymph node or liver FNA cytology specimens. In fact, of the lymph node FNAs performed in adults, the most common diagnosis is metastatic carcinoma. When evaluating a metastatic carcinoma by FNA, it is helpful to have cell block material for immunohistochemical stains that can help to determine the site of origin of the tumor, particularly in those patients without a history of malignancy. For example, an adenocarcinoma showing positivity for thyroid transcription factor-1 (TTF1) is likely to be of lung origin (Figure 23). In patients with a prior malignancy, the morphology and immunocytochemical profile can be compared to determine if the tumors are similar or not. A combination of careful cytomorphological evaluation and ancillary study results can help to determine the site of origin of a metastasis diagnosed by FNA.

Non-epithelial tumors that can also be seen include hematolymphoid proliferations, melanomas, sarcomas, germ cell tumors, mesotheliomas, and others (Figure 19). These tumors are typically less common than metastatic carcinomas and can morphologically overlap with carcinomas; thus, the use of ancillary studies is often crucial, considering the management differs.

**Amyloidosis**

Amyloid is an amorphous, fibrillar protein that is insoluble and can deposit in a variety of tissues to disrupt normal tissue function and create a spectrum of abnormalities, referred to as amyloidosis. Amyloid can be detected with a special stain, Congo Red, where the amyloid will appear pink and demonstrate apple-green birefringence upon polarization (Figure 25). FNA of the abdominal fat pad to acquire vascularized fragments of adipose tissue is a helpful diagnostic test for systemic amyloidosis. The FNA material can be used for confirmatory Congo Red staining, in addition to immunohistochemical stains for further subtyping. In studies analyzing abdominal fat pad, FNA has been shown to have a high specificity, negative predictive value, and positive predictive value. The sensitivity is variable from about 60% to 100%, depending on the diagnostic criteria used in different institutions, the experience of the interpreter, and the prevalence of amyloidosis.

**Conclusions**

This article demonstrates the importance of FNA biopsies in the armamentarium available to patients and clinicians for establishing a diagnosis. Its minimally invasive nature, along
with the low risk of complications, high accuracy, and ability to provide quick answers, makes it a helpful tool. The diagnostic performance of FNA is optimized with trained and experienced cytopathologists, in addition to the presence of high-quality specimens with material for ancillary studies. It is particularly helpful in anxious patients, poor surgical candidates, lesions with a low risk for malignancy, and infections, and in nonsurgical malignancies, where it can alleviate anxiety, assist clinicians in excluding certain diagnostic possibilities, and facilitate clinical management of the patient. Furthermore, in today’s era of personalized medicine, FNA cytology provides a way to acquire small tissue samples that can be used to get diagnostic, prognostic, or predictive information that is essential for patient care.


**Relevant Websites**


Non-Print Items

Abstract:
Fine needle aspiration (FNA) biopsies utilize a thin needle for acquiring cellular material from a lesion or mass in the body for a diagnostic purpose. It is an important diagnostic modality for superficial and deep lesions, because it is minimally invasive, safe, quick, accurate, and cost effective. This article reviews the role of FNA cytology, its advantages and limitations, and the technical aspects of the procedure. Important points are illustrated in a variety of diagrams, images, and supplementary material (e.g., virtual slide and animation). In addition, the approach to evaluating FNA cytology and its diagnostic use in a variety of different lesions is also discussed. The article provides an in-depth overview of FNA cytology and its current role in diagnosis.

Keywords: Aspiration; Cells; Cytology; Cytopathology; Fine needle aspiration; Image guided; Liquid-based cytology; Palpation; Pathology; Slide preparation; Smear; Technique

Multimedia Components:
Animation 1: Demonstration of the technical aspects of the FNA procedure, including aspiration, expulsion of the material, smearing, staining, and interpretation.

Digital slides 1 and 2: Aspirate smears from a lymph node FNA in a patient with a history of malignant melanoma demonstrating metastatic malignant melanoma. The aspirate is cellular and shows numerous discohesive malignant cells with eccentrically placed nuclei, intranuclear inclusions, prominent nucleoli, and occasional binucleation. In addition, the cytoplasm is moderate-to-abundant and has dusty pigmentation. The cells show significant pleomorphism, indicating that a malignant process is occurring and the overall cytomorphological features are consistent with a metastatic malignant melanoma.

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Molecular Imaging: Concepts and Applications in Cardiovascular Disease

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**Glossary**

18F-fluorodeoxyglucose (FDG) A radiolabeled glucose analog detectable by PET imaging that identifies tissues exhibiting high metabolism/inflammation.

Molecular imaging An imaging strategy that uses cellular and molecular targets to identify biological processes in living subjects.

Optical coherence tomography (OCT) A near-infrared light-based optical imaging modality for high-resolution (micrometer) imaging in biological tissues.

**Abbreviations**

**111**In-RP782 111-Indium RP782
ApoE/– Apolipoprotein E-deficient
CAD Coronary artery disease
CEA Carotid endarterectomy
CT Computed tomography
FDG 18F-Fluorodeoxyglucose
ICG Indocyanine green
IVFM Intravital fluorescence microscopy
IVUS Intravascular ultrasound
LAD Left anterior descending coronary artery
MI Myocardial infarction
MMP Matrix metalloproteinase
MRI Magnetic resonance imaging
NaF 18F-Sodium fluoride
NIRF Near-infrared fluorescence
OCT Optical coherence tomography
PET Positron emission tomography
RGD Arginine–glycine–aspartic acid
SNR Signal-to-noise ratio
SPECT Single-photon emission computed tomography
TBR Target-to-background ratio
USPIO Ultrasmall superparamagnetic particles of iron oxide

**Introduction**

As biological pathways and molecular therapeutic targets continue to be elucidated, there is an ongoing need to extend this knowledge into clinical practice. The field of molecular imaging aims to exploit these advances in living subjects through imaging agents tagged with nanoparticles or small molecules that target specific cellular and molecular processes. The added biological information gained from molecular imaging studies has great potential to augment clinical diagnosis, prognosis, and treatment strategies currently based on anatomical and physiological imaging. Furthermore, molecular imaging approaches can assess the biological efficacy of pharmacotherapies in vivo, facilitating personalized medicine and enabling smaller-scale clinical trials that could rapidly determine therapeutic response. While molecular imaging has been applied and clinically translated primarily in the fields of oncology, neurology, and cardiovascular disease (CVD), it is anticipated that molecular imaging will offer utility for many other clinical arenas. Here, we illustrate important fundamentals and limitations of molecular imaging studies, with specific examples of promising translational and clinical molecular imaging applications in CVD.

**Basic Principles of Molecular Imaging**

**Fundamentals**

Molecular imaging seeks to specifically target and report on in vivo aspects of biology. To achieve this aim, first, an accessible molecular target must be identified that involves the biological process of interest. Ideal molecular targets are those that are abundant, often reside on the cell surface or are amenable to internalization/amplification strategies, and are involved in key biological pathways with unique molecular signatures. Molecular imaging targets expressed at low tissue levels can be problematic; however, amplification schemes using enzymes, receptor internalization, or reporter genes may sufficiently increase the target signal so that adequate detection is possible. Access to DNA, RNA, and other intracellular proteins is possible but even more challenging, thus making extracellular targets preferable as they avoid issues of charge, size, and...
Once a desired target is selected, a high-affinity ligand that binds the target is required for selective and specific molecular detection. In most instances, the binding ligand is chosen to interact with the cell or molecule without interrupting its native function; however, in certain cases, pathway disruption may be a desired property, especially for ‘theranostic’ approaches. To maintain a high imaging signal-to-noise ratio (SNR), the ligand must engage specifically with the binding partner while minimizing nonspecific binding to surrounding tissue elements. The most widely recognized example is the antibody–antigen complex; however, the relatively large size of antibodies tends to limit their targeting efficiency in most molecular imaging applications. Instead, small peptides or nanoparticles are fabricated to interact with a specific binding site. Smaller ligands have the added advantage of greater surface coating density when bound to an imaging agent, which can markedly increase the targeting affinity, leading to improved SNR.

Following target and ligand selection, the next fundamental challenge involves choosing an appropriate imaging moiety to conjugate to the targeting ligand. The imaging moiety must be capable of durable linkage to the high-affinity detection ligand and still maintain advantageous biopharmacokinetic properties. Advances in bioengineering have enabled more complex imaging agent designs. Examples include ‘activatable’ agents that can report selectively on tissue enzyme activity, multimodality imaging probes that can read out in the positron emission tomography (PET), magnetic resonance imaging (MRI), and optical domains, and theranostic particles that are capable of simultaneous diagnostic imaging and therapeutic drug delivery. As the choice of imaging agent determines the necessary detection imaging platform, the relative merits of each imaging modality must also be considered in detail.

### Imaging Modalities

Imaging platforms used in molecular imaging studies have particular advantages and disadvantages (Table 1). Spatial and temporal resolutions, sensitivity to molecular imaging agents, depth of field, radiation exposure, and cost represent a few examples of the many important considerations that must be weighed for each molecular imaging application. Presently, clinical molecular imaging strategies rely primarily on agents designed for nuclear and MRI platforms, although computed tomography (CT), ultrasound, and optical approaches continue to gain traction. Stand-alone nuclear imaging with single-photon emission computed tomography (SPECT) or PET offers inherently high sensitivity, but with relatively low spatial resolution that decreases the ability to precisely localize the origin of the molecular signal. Comparatively, MRI exhibits greater spatial resolution but is several orders of magnitude less sensitive for imaging agent detection. As with multimodality molecular imaging agents, combining different modalities into a hybrid imaging approach such as SPECT/CT, PET/CT, or PET/MRI can improve overall diagnostic performance. Other molecular imaging modalities, such as ultrasound with ligand-coated microbubbles and optical-sensing devices with fluorescently labeled particles, offer good imaging sensitivity and high-resolution capabilities at relatively low cost. In addition, ultrasound and optical approaches require no exposure to ionizing radiation, an important clinical consideration in human subjects given the potential need for repeated imaging studies to follow disease progression and response to treatment.

### Molecular Imaging Applications in CVD

Molecular imaging continues to expand within all areas of CVD, due to a growing number of FDA-approved clinical imaging probes and many promising, highly translatable preclinical agents (Table 2). In particular, significant insights within the areas of atherosclerosis, thrombosis, vascular injury, and aneurysm have been realized. To achieve this progress, cardiovascular molecular imaging investigators have overcome important obstacles related to cardiac and respiratory motion for myocardial imaging, small vessel size for coronary applications, and the influence of flowing blood. As more agents move to the clinical arena in the coming years, the impact of cardiovascular molecular imaging is likely to grow. As leading examples, here, we highlight advances in molecular imaging of atherosclerosis, thrombosis, aneurysm, and vascular injury.

### Atherosclerosis

Atherosclerotic vascular disease, consisting of lipid- and macrophage-rich plaques resident in the vessel wall, offers...

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**Table 1** Important characteristics of clinical molecular imaging modalities

<table>
<thead>
<tr>
<th>Technique</th>
<th>Agents</th>
<th>Sensitivity</th>
<th>Resolution</th>
<th>Depth</th>
<th>Scan time</th>
<th>Cost</th>
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</thead>
<tbody>
<tr>
<td>Positron emission tomography (PET)</td>
<td>Radionuclides</td>
<td>High</td>
<td>Millimeter</td>
<td>(\infty)</td>
<td>Minutes</td>
<td>$$$</td>
</tr>
<tr>
<td>Single-photon emission computed tomography (SPECT)</td>
<td>Radionuclides</td>
<td>High</td>
<td>Millimeter</td>
<td>(\infty)</td>
<td>Minutes</td>
<td>$$</td>
</tr>
<tr>
<td>Magnetic resonance imaging (MRI)</td>
<td>Paramagnetic and magnetic particles</td>
<td>Medium</td>
<td>Micrometer</td>
<td>(\infty)</td>
<td>Minutes</td>
<td>$$</td>
</tr>
<tr>
<td>Computed tomography (CT)</td>
<td>Iodine compounds</td>
<td>Low</td>
<td>Micrometer</td>
<td>(\infty)</td>
<td>Seconds</td>
<td>$$</td>
</tr>
<tr>
<td>Contrast-enhanced ultrasound (CEU)</td>
<td>Microbubbles</td>
<td>Medium</td>
<td>Micrometer</td>
<td>Centimeter</td>
<td>Minutes</td>
<td>$$</td>
</tr>
<tr>
<td>Fluorescence reflectance imaging (FRI)</td>
<td>Fluorophores, photoproteins</td>
<td>High</td>
<td>Millimeter</td>
<td>Millimeter</td>
<td>Seconds</td>
<td>$</td>
</tr>
<tr>
<td>Near-infrared fluorescence imaging (NIRF)</td>
<td>Near-infrared fluorophores</td>
<td>High</td>
<td>Micrometer</td>
<td>Millimeter</td>
<td>Seconds</td>
<td>$$</td>
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Currently, two imaging agents dominate clinical atherosclerosis molecular imaging: 18F-fluorodeoxyglucose (FDG) for PET imaging and ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles for MRI. Both FDG and USPIO target plaque inflammation, but, due to their lower resolution (PET) or lower sensitivity (MRI), are best suited for imaging large-diameter arterial beds such as the carotid, aorta, and iliofemoral vessels. Because of their smaller caliber, coronary arteries are less accessible to FDG and USPIO imaging approaches; however, high-resolution intravascular optical molecular imaging catheters that can detect inflammation are emerging into the clinical arena. On the horizon, there are many new atherosclerosis molecular imaging agents currently in development with significant promise for clinical use (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Agent</th>
<th>Modality</th>
<th>Primary Target</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDG (18F-fluorodeoxyglucose)</td>
<td>PET</td>
<td>Glucose transporter-1, hexokinase</td>
<td>Atherosclerosis, aneurysm, vascular injury (metabolism)</td>
</tr>
<tr>
<td>USPIO (ultrasmall superparamagnetic iron oxide)</td>
<td>MRI</td>
<td>Macrophages</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>ProSense</td>
<td>NIRF, FRI</td>
<td>Cysteine protease activity Macrophages</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>MMPSense</td>
<td>NIRF, FRI</td>
<td>Matrix metalloproteinas</td>
<td>Atherosclerosis, aneurysm, vascular injury (inflammation)</td>
</tr>
<tr>
<td>P947</td>
<td>MRI</td>
<td>Matric metalloproteinas</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>99mTc-RP805</td>
<td>SPECT</td>
<td>Matric metalloproteinas</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>111In-RP782</td>
<td>SPECT</td>
<td>Matric metalloproteinas</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>VCA-M1 microbubbles</td>
<td>CEU</td>
<td>VCAM-1</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>18F-Galacto-RGD</td>
<td>NIRF, FRI</td>
<td>Lipids/macrophages</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>NaF (sodium 18F-fluoride)</td>
<td>PET</td>
<td>Integrins/endothelium 3 integrins/endothelium</td>
<td>Atherosclerosis (inflammation)</td>
</tr>
<tr>
<td>OsteoSense</td>
<td>NIRF, FRI</td>
<td>Hydroxyapatite</td>
<td>Atherosclerosis (calcification)</td>
</tr>
<tr>
<td>99mTc-annexin A5</td>
<td>SPECT</td>
<td>Annexin-A5/macrophages</td>
<td>Atherosclerosis (apoptosis)</td>
</tr>
<tr>
<td>EP-2104R</td>
<td>MRI</td>
<td>Fibrin</td>
<td>Thrombosis (coagulation)</td>
</tr>
<tr>
<td>RTP11-Gy/7</td>
<td>NIRF, FRI</td>
<td>Fibrin</td>
<td>Thrombosis (coagulation)</td>
</tr>
<tr>
<td>Activated factor XIII (FXIIIa)</td>
<td>SPECT, NIRF</td>
<td>FXIIIa activity</td>
<td>Thrombosis (coagulation)</td>
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Driven by activated monocytes and macrophages, atherosclerotic plaque inflammation is a potent stimulant for plaque growth and instability. Autopsy specimens of sudden cardiac death patients demonstrate extensive macrophage infiltration in culprit plaques, supporting inflammation as a primary determinant of plaque rupture. Therefore, inflammation is a high-risk plaque marker that may aid in risk stratification of otherwise anatomically similar plaques and guide development of future plaque-stabilizing therapies. An observational study of 932 asymptomatic patients imaged with hybrid FDG PET/CT supports this principle, where FDG-identified atherosclerosis inflammation in the aorta, iliac, and carotid arteries was four times more predictive of future vascular events compared to CT calcification. While FDG PET and USPIO MRI are the most extensively studied plaque inflammation reporters, promising new CT and optical agents, among others, are being investigated.

**18F-Fluorodeoxyglucose**

FDG is a glucose analog that accumulates in energy-consuming cells, frequently used in cancer patients to identify metabolically active malignant cells. In CVD, FDG is increased at sites of inflammation, where histopathology demonstrates correlation with tissue macrophages and proinflammatory molecules including interleukins and proteolytic enzymes. Traversing normal glucose pathways, FDG concentrates in the cytosol by active transport mechanisms, where it becomes intracellularly trapped following hexokinase phosphorylation. Intracellularly sequestered FDG emits positrons with a 110 min half-life that can then be detected by PET imaging systems. As FDG is widely available clinically, its use in molecular imaging has substantially grown. However, due to spatial resolution limitations, FDG PET must be performed with hybrid CT or MRI anatomical imaging to allow sufficient tissue localization, leading to increased radiation exposure and longer scan times, respectively.
Ultrasmall superparamagnetic particles of iron oxide

USPIO nanoparticles are formed from a 3 nm superparamagnetic iron oxide core externally coated with dextran resulting in ~30 nm hydrodynamic nanoparticles that can be ingested by professional phagocytes. The magnetic core enables MRI detection by influencing T2- and T2*-weighted image sequences, while the dextran coating promotes phagocytosis by macrophages allowing inflammatory cell detection in atherosclerotic plaques. As long-circulating USPIO exhibit prolonged blood pharmacokinetics and require time to infiltrate target tissues, they are administered 24–48 h prior to MRI, thus limiting their use for acute diagnostic imaging. Furthermore, USPIO create ‘negative’ contrast, detected as dark signal voids on MRI images, which can sometimes be challenging to discriminate from vessel wall background signal. Comparatively, MRI has better spatial resolution than PET but is significantly less sensitive in detecting targeted imaging agents.

Large-caliber arteries

Larger arteries, such as the aorta, carotid, and iliofemoral vessels, are susceptible to extensive atherosclerotic plaque burden. Clinical USPIO and FDG are well suited for inflammation detection in large arteries and have uncovered important diagnostic and therapeutic insights in humans. In particular, the response of atherosclerotic plaque inflammation to statin therapy, which not only primarily lowers circulating lipid levels but also invokes anti-inflammatory and plaque stabilizing effects, has been tested by noninvasive molecular imaging in several human studies.

Enhanced carotid atherosclerotic plaque inflammation by FDG PET and USPIO MRI has been demonstrated in a variety of small human clinical trials. Notably, carotid USPIO uptake does not correlate with stenosis severity in asymptomatic patients, suggesting that inflammation and the degree of lumen obstruction are uncoupled. In addition, subjects with symptomatic carotid plaques (i.e., recent neurological event in the ipsilateral carotid artery territory) exhibited greater USPIO inflammation compared to those with asymptomatic stenosis. In FDG studies, PET identified FDG inflammation in 30% of carotid plaques from a consecutive series of 100 patients. Consistent with the USPIO studies, increased FDG uptake was greater in symptomatic compared to asymptomatic carotid endarterectomy (CEA) patients. Application of FDG PET has also been extended to patients with the metabolic syndrome and diabetes mellitus, who demonstrated increased FDG carotid plaque inflammation compared to control subjects.

Statin efficacy

In the Atheroma trial (Atorvastatin Therapy: Effects on Reduction of Macrophage Activity), 47 patients with carotid artery stenosis >40% by duplex ultrasound were randomized to high-dose (80 mg) or low-dose (10 mg) atorvastatin. To define changes in carotid plaque inflammation, each group underwent serial USPIO MRI at baseline and then after 6 and 12 weeks statin therapy (Figure 1). In the high-dose statin

![Figure 1](image-url) Molecular MRI of atherosclerosis inflammation in statin-treated patients. Common carotid artery T2*-weighted MRI images pre- and post-USPIO administration at (a and b) 0, (c and d) 6, and (e and f) 12 weeks of high-dose atorvastatin pharmacotherapy. Baseline USPIO (b, yellow arrowhead) diminishes over time demonstrated by absence of focal signal loss (d and f, blue arrowheads) related to decreased local inflammation. Before USPIO reinjection, the background signal from prior USPIO administration is minimal (c and e, red arrowheads). Reproduced from Tang, T.Y., et al., 2009. The Atheroma (Atorvastatin Therapy: Effects on Reduction of Macrophage Activity) Study. Evaluation using ultrasmall superparamagnetic iron oxide-enhanced magnetic resonance imaging in carotid disease. J. Am. Coll. Cardiol. 53, 2039–2050, with permission.
group, low-density lipoprotein (LDL) levels fell 29%, and there was an associated decrease in USPIO-identified carotid inflammation at both 6 and 12 weeks (change in signal intensity 0.13 and 0.20, respectively; \( p < 0.001 \) for both timepoints). Transcranial Doppler imaging in the high-dose subjects demonstrated a reduction in carotid artery emboli. In contrast, the low-dose group experienced no significant change in serum LDL, carotid USPIO signal intensity, or Doppler microembolic events. This finding highlights how molecular imaging can provide biological insights into the effect of differential statin doses.

Carotid artery FDG-defined inflammatory activity is also diminished in clinical statin therapy trials. A retrospective review of 200 asymptomatic patients determined that statin treatment correlated with fewer FDG-positive carotid plaques. In a prospective study, 43 patients found to have carotid FDG activity on PET cancer screening were treated with daily simvastatin therapy titrated to goal LDL <130 mg dL\(^{-1}\) for 3 months. Compared to dietary changes alone, daily simvastatin significantly decreased carotid FDG signal (Figure 2). Taken together, noninvasive human FDG and USPIO studies can identify carotid atherosclerotic inflammation and assess treatment response, demonstrating the potential clinical utility of a molecular imaging approach.

Coronary arteries

Molecular imaging of the coronary arteries presents many technical challenges. In addition to cardiac and respiratory motions, the small diameter of the coronary vessels requires performance imaging systems that can provide both high spatial and temporal resolution and high sensitivity. Although demanding, coronary artery molecular imaging continues to advance through noninvasive and invasive techniques.

Despite the relatively low resolution of PET (3 mm isotropic), FDG PET has been preliminarily applied to image coronary artery inflammation. FDG activity measured in the proximal left anterior descending (LAD) coronary artery correlated with coronary artery disease (CAD), hypertension, and body mass index in 292 asymptomatic patients; however, the high FDG activity in the surrounding myocardium precluded analysis in nearly half of the subjects. The use of dietary myocardial metabolic suppression protocols that incorporate low-carbohydrate, high-fat meals can improve the yield of analyzable FDG coronary segments. In one study of 25 patients with stable or unstable CAD, dietary myocardial suppression allowed left main coronary artery FDG analysis with 96% success, including 89% of implanted coronary stents. Compared to stable plaques, patients with unstable coronary syndromes demonstrated enhanced left main coronary artery FDG uptake (plaque target-to-background ratio 2.48 vs. 2.00, \( p = 0.03 \); Figure 3). With further optimization, reproducible FDG interrogation of the proximal epicardial coronary arteries may be possible with FDG PET; however, analysis in more distal segments will likely remain technically difficult.

Atherosclerotic calcification has also been interrogated in human coronary arteries with sodium \[^{18}\mathrm{F}\text{-fluoride (NaF)}\), a PET imaging agent that identifies sites of active mineralization (Figure 4). One hundred and nineteen patients imaged with NaF PET revealed enhanced NaF uptake in those subjects with cardiovascular risk factors. When compared to CT coronary calcium scoring, NaF PET mineralization signal correlated well with the CT results (\( \rho = 0.65, \ p < 0.001 \)), but not with FDG PET likely due to limitations differentiating coronary artery FDG signal from the high background myocardial FDG activity. NaF detection of calcium deposition in plaques may thus signify atheroma at different stages of remodeling and maturation, although its ability to contribute clinical information beyond that already available through CT coronary artery calcium scoring is yet to be established.

While invasive, intravascular molecular imaging offers the best option for high-sensitivity \textit{in vivo} coronary plaque biology imaging. Prototype molecular imaging catheters have been developed for through-blood imaging based on near-infrared fluorescence (NIRF). Intravascular NIRF catheters can successfully report on inflammation in human coronary-sized rabbit aorta and iliac arteries. Following intravenous administration of ProSense VM110, a protease-activatable NIRF imaging agent that reports on tissue protease inflammation (Figure 5), real-time catheter pullbacks revealed enhanced NIRF inflammation within atherosclerotic plaques identified by concomitant intravascular ultrasound (IVUS) imaging (Figure 6). New catheter designs that combine sensitive NIRF imaging fibers and detailed anatomical imaging with optical frequency domain imaging, a high speed form of optical coherence tomography (OCT), can collect simultaneous molecular and microstructural information for precise coregistered imaging (Figure 7). Intravascular MRI catheters have also been described, which with further improvements in temporal acquisition could be used for coronary molecular imaging. Although less applicable to broad populations than noninvasive strategies, intravascular coronary artery molecular imaging strategies are likely to translate to the clinical arena to guide diagnosis and treatment in high-risk CAD patients.
Novel agents for atherosclerotic plaque inflammation molecular imaging continue to be designed and tested, increasing the potential pool of clinically translatable agents and driving innovation that can be applied to new imaging strategies. In particular, multimodality probes that incorporate multiple imaging reporters are becoming available, such as a nanoparticle that can be detected by MRI, PET, and NIRF. This tri-modality agent consists of a 20 nm iron oxide core coupled to a 680 nm fluorochrome and the radiotracer $^{64}$Cu, with a dextran surface coating that promotes macrophage ingestion. By enabling imaging on different platforms, multimodality agents allow cross-sectional target verification and provide great flexibility that may benefit specific molecular imaging applications.

Macrophage-derived proteases are high-impact atherosclerosis inflammation molecular imaging targets as active proteolysis identifies inflamed, potentially vulnerable, plaques. As previously discussed, plaque cysteine protease activity has been demonstrated with ProSense, a protease-activated NIRF reporter for optical imaging systems that targets cathepsins. Matrix metalloproteinases (MMPs) are also significant contributors to plaque inflammation that have been successfully visualized with the MRI gadolinium chelate $^{99m}$Tc-MMP and $^{111}$In-RP782.

Atherosclerosis inflammation is linked to endothelial injury and dysfunction, which increases leukocyte rolling and adhesion as a result of proinflammatory endothelial surface receptor upregulation. Although the endothelium is readily accessible to intravascularly administered probes, challenges to endothelial molecular imaging include sufficient agent binding within the high shear stress blood flow environment and relatively low signal density from the single-cell thick layer. Despite these obstacles, molecular imaging agents can detect endothelial inflammatory receptors, including VCAM-1-targeted microbubbles for contrast-enhanced ultrasound, nanoparticles for NIRF and MRI, and $^{18}$F-labeled tetrapeptide affinity ligand for PET. In addition, probes for the surface glycopolypeptides E- and P-selectin have been developed including MRI microparticles of iron oxide and CT iodinated liposomes.

Figure 3  Clinical coronary artery FDG PET/CT imaging. (a) A patient treated for an acute coronary syndrome (ACS) with percutaneous coronary intervention revealed enhanced FDG inflammation in the stented culprit lesion (arrows) and the left main (LM) coronary artery (hatched arrows). In contrast, coronary FDG enhancement was less in representative patients with (b) stable coronary disease treated electively with stent placement or (c) that had undergone prior stent implantation. (d) Another patient with ACS also revealed enhanced coronary FDG signal at the LM bifurcation. Reproduced from Rogers, I.S., et al., 2010. Feasibility of FDG imaging of the coronary arteries: comparison between acute coronary syndrome and stable angina. JACC Cardiovasc. Imag. 3, 388–397, with permission.
Neovascularization

Growth factors secreted from atheroma constituents stimulate formation of new endothelial-lined vessels, a feature found in unstable plaques that may promote intraplaque hemorrhage. Neovessel endothelial cells are tethered to the extracellular matrix in part by $\alpha_\text{v} \beta_3$ integrin receptors, which are detectable by molecular imaging agents that incorporate arginine–glycine–aspartic acid (RGD) peptide sequences. Examples of preclinical $\alpha_\text{v} \beta_3$ integrin molecular imaging agents include $^{18}$F PET labeling and MRI-based paramagnetic nanoparticles or gadofluorine M.

Figure 4 Sodium $^{18}$F-fluoride (NaF) PET imaging of coronary artery calcification. NaF was absent in (a) control subjects without coronary artery calcification and (b) select subjects with extensive CT coronary calcification that likely represented advanced plaques. (c and d) In plaques with active mineralization, NaF uptake colocalized with calcified left anterior descending (LAD) coronary artery disease on CT imaging. (e) Example of a patient with an acute coronary syndrome revealed NaF uptake in the calcified proximal right coronary artery (RCA) culprit lesion, but not the heavily calcified LAD. (f) Coronary angiography in the patient from (e) demonstrated a proximal RCA ruptured plaque with thrombus formation. Reproduced from Dweck, M.R., et al., 2012. Coronary arterial $^{18}$F-sodium fluoride uptake: a novel marker of plaque biology. J. Am. Coll. Cardiol. 59, 1539–1548, with permission.
Theranostics
Molecular imaging probes with both diagnostic and therapeutic capabilities, termed theranostics, are greatly sought. Integrin-targeted agents to detect and simultaneously treat neovessels have been developed by coupling the anti-microvessel compound fumagillin to $\alpha_v\beta_3$ MRI magnetic nanoparticles. In atherosclerotic rabbits administered with fumagillin theranostic magnetic nanoparticles, MRI plaque $\alpha_v\beta_3$ neovascularization significantly lessened at 4 weeks. Theranostic approaches have also shown promise for targeted macrophage detection and local destruction through photodynamic therapy with near-infrared fluorescent light using fluorescent nanoparticles or protease sensors. In addition to the single-agent convenience of a theranostic approach, molecularly targeted pharmacotherapy has the potential to limit systemic drug toxicity.

Calcification
Chronic inflammation triggers calcium deposition, which may manifest as atheroma microcalcifications linked histopathologically to plaque rupture. In addition to plaque risk stratification, identification of early-stage calcium formation may be reversible with medical therapy, as opposed to late-stage calcification, which can only be effectively treated by endovascular intervention or surgical resection. Calcification-specific near-infrared fluorescence (NIRF) optical molecular imaging agents have been developed that report on osteoblast hydroxyapatite deposition, a key bone mineral component. Using OsteoSense 750, a hydroxyapatite-specific NIRF probe (excitation 750 nm), and macrophage-targeted fluorescent nanoparticles (excitation 680 nm) to identify plaque inflammation, simultaneous multichannel intravital fluorescence microscopy (IVFM) in atherosclerotic mice investigated associations between early microscopic calcification and carotid plaque inflammation. Multichannel NIRF IVFM demonstrated early calcification tracked with macrophage accumulation, which was invisible to standard histopathology stains but confirmed by direct electron microscopic visualization of hydroxyapatite nanocrystals (Figure 8). Furthermore, incorporating statins into the animal chow for 10 weeks decreased both NIRF inflammation and calcification. A close relationship between inflammation and
Calcification was supported by a similar IVFM study in an atherosclerotic mouse model with chronic renal failure known to accelerate vascular calcification, where OsteoSense-detected calcification also increased in relation to a co-injected inflammatory NIRF protease reporter for cathepsin S.

**Apoptosis**

Cell death within atheroma leads to plaque expansion and instability. Apoptosis is an important contributor to intraplaque cell loss, mediated by the caspase enzyme family, which exposes cell-surface phosphatidylserine residues that bind
 annexin proteins. NIRF caspase activity-based probes for optical apoptosis imaging are available. In addition, development of radionuclide-labeled annexin molecular imaging agents for SPECT, such as $^{99m}$Tc-annexin and $^{111}$In-annexin, has allowed *in vivo* apoptosis visualization. In atherosclerotic animal models, SPECT $^{99m}$Tc-annexin plaque activity was diminished in rabbits administered with caspase inhibitors, and hypercholesterolemic mice exhibited enriched plaque $^{99m}$Tc-annexin SPECT apoptosis signals compared to controls. $^{99m}$Tc-annexin SPECT apoptosis imaging has also been translated to humans in a pilot study of four patients with carotid stenosis and prior transient ischemic attack. Two patients with recent neurological events only 3–4 days before imaging demonstrated enhanced $^{99m}$Tc-annexin in culprit carotid plaques without uptake in the contralateral artery. In comparison, the patients that experienced remote events >3 months prior revealed no significant $^{99m}$Tc-annexin uptake. Histopathology of excised CEA specimens corroborated increased vulnerable plaque features in the patients with recent events, including macrophage infiltration, intraplaque hemorrhage, and annexin staining.

**Thrombosis**

Thrombosis results in vessel occlusion and/or embolization to vital organs and is a significant cause of adverse cardiovascular events including MI, deep vein thrombosis, pulmonary embolism, stroke, and acute limb ischemia, among others. Activated plasma clotting factors and platelets are key thrombotic cellular elements, providing a rich pool of potential molecular imaging targets. Current clinical anatomical imaging modalities, including ultrasound, CT, and MRI, can identify most larger thrombi effectively but lack information on the relative biological composition of the clot (fibrin- or platelet-rich) that may influence therapeutic choices and furthermore are unable to discriminate whether the thrombus is acute or chronic. Smaller microscopic thrombi, related to atherosclerotic plaque rupture or intraplaque hemorrhage, are more challenging to detect with current untargeted imaging techniques due to resolution constraints and imaging and motion artifacts. To address these deficiencies, thrombosis molecular imaging aims to augment anatomical imaging with supplementary biological readout, ideally through noninvasive strategies.
Molecular imaging agents for thrombus detection are progressing to the clinic, such as the fibrin-targeted peptide EP-2104R. Each EP-2104R peptide is coupled to four gadolinium molecules enabling MRI detection and is administered 2–6 h prior to imaging to allow time for blood pool agent washout. A phase II multicenter clinical trial of EP-2104R has been completed in patients with newly detected arterial or venous thrombosis. Of the 52 thrombi discovered by either ultrasound, CT, or MRI, noncontrast MRI alone identified 56% of clots. The addition of EP-2104R revealed another 7 clots, increasing the overall yield to 69% (Figure 9). While a significant number of thrombi were not visualized even with EP-2104R, potentially due to restricted binding sites in densely cross-linked clots, overall EP-2104R was safe with rare adverse reactions. More recently, the peptide backbone of this agent has been linked to an NIR fluorescent reporter for optical imaging (Figure 10), enabling the potential for high-resolution clinical intravascular catheter-based imaging of fibrin deposition on coronary stents.

Apart from EP-2104R, new molecular imaging agents formulated to detect cellular elements and clotting proteins involved in thrombosis pathways are emerging. Platelet-rich arterial clots in pigs have been successfully imaged in real time with NIRF after injecting platelets directly labeled with a fluorescent compound. The activated form of the platelet surface receptor glycoprotein IIb/IIIa has also been detected by MRI with antibody-coated iron oxide microparticles and can be followed noninvasively to monitor clot resolution after thrombolysis. Clotting factors are prime targets, such as a NIRF thrombin activity peptide biosensor tested in acute thrombi and as well as in atheroma. Activated transglutaminase factor XIII (FXIIIa), a coagulation protein important for fibrin cross-linking and incorporation of circulating α2-antiplasmin into acute thrombi, is another investigated molecular imaging target. By IVFM, NIRF-labeled FXIIIa has been used to identify cerebral sinus vein thrombi and differentiate acute from subacute peripheral clots. Dual-modality MRI and NIRF FXIIIa imaging with a gadolinium-linked fluorescent α2-antiplasmin peptide sequence could detect acute thrombus formation 90 min after induction in mice but was insensitive to identify thrombi > 24 h old. In the future, molecular imaging probes for specific in vivo thrombus detection are highly likely to become clinically available and offer various strategic reporters to identify clot composition and resolution in individualized patients that may significantly alter treatment strategies.
Aneurysmal Disease and Vascular Injury

Vascular injury may occur secondary to local inflammation, atherosclerosis, or mechanical trauma and can lead to progressive atherosclerotic-type stenosis characterized by excessive neointimal formation due to smooth muscle cell infiltration or, in certain cases, focal weakening of the vessel wall infrastructure leading to aneurysm. Aneurysms can affect many arterial beds, including the smaller coronary and cerebrovascular arteries and the larger aorta, carotids, and iliofemoral vessels. In each case, local inflammation is believed to be a strong driver of disease progression. In atherosclerotic-based aneurysmal disease, neovascularization is a high-risk phenotype that may signify lesion instability and subsequent progression. In the case of aneurysms, progressive vascular dilatation and local tissue instability may lead to frank vessel wall rupture, hemorrhagic shock, and death. Molecular imaging is poised to illuminate hallmark properties of vascular injury and aneurysm formation, propagation, and risk stratification.

Inflammation

FDG imaging of aneurysm metabolism/inflammation has illuminated the contribution of macrophage activity in predicting aneurysm complications (Figure 11). Histopathology following surgical resection revealed local markers of tissue instability, including elastin degradation, MMP activity, and...
macrophage infiltration. These markers correlated with sites of enhanced FDG uptake. A 14-patient study of infrarenal abdominal aortic aneurysm (AAA) revealed enhanced FDG signal in subjects with concomitant inflammation on CT imaging. In contrast, CT calcification did not track with AAA FDG activity, implying that calcification may identify less clinically active aneurysms. Finally, FDG PET also has been demonstrated in a small 18-patient study to identify sites of aortic dissection, with the ability to differentiate FDG-enhancing acute from less FDG-avid chronic dissections. Since treatment strategies for aortic dissection in the absence of confounding clinical complications may be strongly influenced by the chronicity of the dissected vessel, FDG PET imaging may offer substantial incremental data to influence surgical treatment decisions.

New inflammation-targeted molecular imaging agents may hold promise for future clinical applications. An MRI-sensitive oxidative stress reporter, myeloperoxidase enzyme activity, can detect tissue oxidation in experimental rabbit intracranial aneurysms. MMPs associated with aneurysms can also be
detected with optical NIRF imaging agents that correlate with aneurysm inflammation and subsequent expansive growth. Vascular injury models have similarly detected associations between MMP activity and neointimal formation using the MMP-sensitive SPECT tracer $^{111}$In-RP782. Multimodality macroprobe-targeted magnetofluorescent iron oxide nanoparticles for MRI and optical detection have been further functionalized with FDG for PET imaging and validated to accumulate in inflamed mouse aneurysm models. As additional molecular markers of aneurysm risk become evident, a more refined approach to aneurysm treatment may be possible including earlier therapy for selected patients.

**Neovascularization**

New blood vessel formation in diseased tissues may lead to local hemorrhage, inflammation, instability, and rupture. Local neovascularization has been demonstrated to signify a high-risk phenotype in vascular disease states, and therefore, employing highly specific molecular imaging targets to evaluate neovessel formation has great potential to improve on current clinical risk predictors. In aneurysmal disease, in vivo NIRF optical imaging of the vascular endothelial growth factor (VEGF) receptor, a well-established hallmark of neovessel formation, was found to correlate with ultrasound-determined AAA size. $\alpha_v\beta_3$ integrin receptor expression on endothelial cells forming new blood vessels is also a frequently investigated molecular imaging target. RP748, an $^{111}$In-labeled $\alpha_v\beta_3$ integrin receptor agent for SPECT imaging, revealed enhancement at injured vascular sites in animal models. Furthermore, the degree of neovessel formation associated with the amount of vessel wall remodeling.

**Current Limitations and Future Directions**

Emerging molecular imaging technologies hold great potential to revolutionize our current ability to interrogate clinical disease entities by enabling vast new insights into human disease. In particular, molecular imaging offers the possibility to illuminate high-yield molecular, cellular, and biological targets in vivo in living subjects. In many instances, molecular imaging provides noninvasive reporters that require no ionizing radiation exposure and therefore are highly suited for serial drug-response studies and personalized therapeutics. Given the rapid propagation of available probes, and the recent growth of human clinical investigations, molecular imaging is positioned to greatly enhance our understanding of human physiology and disease. Although highly promising, most clinical molecular imaging investigations remain preliminary and require further extensive validation in larger outcome trials, for example, in cardiovascular applications evaluating associations with major cardiac events (e.g., death, MI, and stroke), before they can be applied to wider populations. Furthermore, for many probes, there exist significant FDA regulatory limits that must be navigated to allow new promising molecular imaging agents to become available for clinical testing. Overall, however, the long-term outlook for clinical molecular imaging is bright and has significant likelihood to offer insights into the risk stratification, treatment-response, and complications related to human disease states.

**Further Reading**

**Basic Principles**


**Atherosclerosis**


**Further Reading**


Tahara, N., Kii, H., Yagishita, S., Mizoguchi, M., Nakaoka, H., Ishibashi, M., et al., 2007. Vascular inflammation evaluated by $^{18}$F-fluorodeoxyglucose positron imaging...
emission tomography is associated with the metabolic syndrome. J. Am. Coll. Cardiol. 49, 1533–1539.


Thrombosis


Aneurysm and Vascular Injury


Relevant Websites

Center for Molecular Imaging Research, Massachusetts General Hospital, Harvard Medical School – http://cmir.mgh.harvard.edu/.


Non-Print Items

Abstract:
Molecular imaging aims to extend current clinical imaging approaches by utilizing targeted agents to illuminate important biology in living subjects. Cellular and molecular processes of interest are identified by bioengineered nanoparticles or small molecules that specifically detect the desired target. Molecular imaging probes have been formulated for readout by a wide range of clinical imaging modalities including magnetic resonance imaging, computed tomography, positron emission tomography, single-photon emission computed tomography, ultrasound, and optical imaging. Overall, molecular imaging technologies are witnessing growing application in the fields of oncology, neurology, and cardiovascular disease (CVD), where they are likely to significantly impact diagnostic and therapeutic decision making. Furthermore, molecular imaging offers the ability to precisely assess the biological response to disease treatment in individual patients over time, opening the door for personalized medicine strategies guided by molecular imaging reporters. This article highlights important principles of molecular imaging and provides examples of molecular imaging applied to important topics in CVD.

Keywords: Aneurysm; Atherosclerosis; Computed tomography; Congestive heart failure; Coronary artery disease; Inflammation; Magnetic resonance imaging; Molecular imaging; Myocardial infarction; Positron emission tomography; Theranostics; Thrombosis; Vascular injury

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Biographical Sketch

Farouc Jaffer is an associate professor of medicine at Harvard Medical School and an attending interventional cardiologist at Massachusetts General Hospital. He is a principal investigator in the MGH Cardiovascular Research Center. His laboratory develops in vivo molecular imaging and theranostic approaches to visualize and treat vascular disease, including atherosclerosis, venous and arterial thrombosis, and vascular injury. A major emphasis is on the development of intravascular biological imaging technology translatable to human coronary arteries. His laboratory has received support from the National Institutes of Health (NIH/NHLBI), Howard Hughes Medical Institute, and American Heart Association.
Eric Osborn is a senior fellow in interventional cardiology at Beth Israel Deaconess Medical Center and Harvard Medical School. He is also a research fellow in the Cardiovascular Research Center at Massachusetts General Hospital and Harvard Medical School in the laboratory of Dr. Farouc Jaffer investigating molecular imaging to illuminate in vivo biology of atherosclerosis, thrombosis, and coronary artery stent healing.