**Lymph node fine needle cytology in the diagnosis of infectious diseases and ancillary techniques**

*Citologia per ago sottile dei linfonodi nella diagnosi delle malattie infettive e tecniche ancillari*

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**INTRODUCTION**

Fine needle cytology is a powerful tool in the diagnosis of infectious lesions. Being minimally invasive, easily performed and well tolerated by the patients, this technique is often used in clinical practice to assess lymph node, skin lung and other organs frequently involved by infectious diseases [1-18]. FN C offers the possibility to make diagnosis on cytomorphological stains, to obtain-deliver material for other ancillary techniques and at the same time it permits to store residual material for future applications. All these advantages allow a rapid diagnosis, an immediate treatment with minimal invasion and costs for the patient. FN C uses a 23 gauge needle with a negative pressure or capillary action to draw cells into the hub of the needle. Direct smears are prepared from material obtained from FN C.

Sampling procedures should be performed by a cytopathologists to allow a correct specimen triage during the procedure, improving accuracy and reducing complications. Specimens can be air-dried or alcohol-fixed for immediate evaluation, to determine both adequacy and most appropriate selection of cytopreparatory and staining methods.

In fact, infectious agents can be identified by cytomorphology with routine or special stains. The rapid on site evaluation (ROSE) (Figure 1) is particularly useful when an infectious disease is suspected since an additional sterile sample can be obtained for the microbiology laboratory. This latter will identify microorganisms and the cytopathology laboratory the cell components and can suggest or sometimes identify the presence of an infectious agents on the basis of some cytological features. In fact morphological diagnosis on direct smears and special stains allows the detection of specific microorganisms, such as fungi, parasites, mycobacteria and viruses. Conventional cytological stains such as Giemsa and Papanicolaou allows the identification of different microorganisms: some families of bacteria, mycobacteria, viruses, fungi and parasitic agents.
fungal hyphae and cytomegalovirus cytoplasmic inclusion are better observed on Giemsa stained smears whereas some fungal hyphae and viral intranuclear inclusion are better observed on Papanicolaou stained smears. In case of suspected specific infection, extra air-dried slide may be stained with auramine, Ziehl Nielsen (Figure 2), Gram and Grocott Methenamine silver stain (GSM).

The best approach is obtained by the combination of previous reported staining methods with ancillary techniques such as PCR, Immunofluorescence (IF), Flow Cytometry (FC), gene rearrangement studies and sometimes philogenetic analyses based on nuclear small subunit RNA sequence alignments [19-22]. This approach allows rapid detection of microorganisms that were previously difficult or impossible to detect by only traditional methods. In this perspective, molecular biological methods for the detection and characterization of microorganisms have revolutionised diagnostic procedures and are now part of clinical practice. This study will focus on the clinical utility of ancillary techniques applied on FNC samples showing how they have aided laboratory diagnosis and management of lymph nodal infectious diseases.

### FUNGI

Most fungi are diagnosed in cytology specimens evaluating their morphologic characteristics, combined with routine (Papanicolaou and Giemsa) and specific coloration methods and with serological-immunologic techniques. Morphological examination of smears allows a rapid detection without lacking of sensitivity and specificity. Most fungal infections usually start in the respiratory tract but may be secondarily identified in lymph nodes and cerebrospinal fluid (CSF) of immunocompromised patients. Some examples of fungi detectable in lymph nodal areas and their specific stain are:

- Cryptococcosis neoformans - which is positive in GSM and its mucopolysaccharidic capsule results positive with mucicarmine stain;
- Blastomycosis dermatitis - identifiable with Papanicolaou and GSM;
- Histoplasmosis capsulatum - identifiable with Giemsa and GSM, not with Papanicolaou
- Penicillium - GSM positive;
- Pneumocystis jiroveci - identifiable with Papanicolaou, Giemsa, GSM and with Immunoperoxidase revelation.

A definitive identification requires the culture of the organism and this is still considered the gold-standard method for the diagnosis of fungal infections, but this procedure requires long times to be performed. The advent of molecular methods has improved sensitivity, specificity and reduced times of performing for diagnostic procedures based on acid-nucleic tests for the detection and the identification of fungal pathogens. Commercially available testing, AccuProbes by Gen-Probe, Inc. relies on the sequence-specific hybridization of a chemiluminescent, single-stranded DNA probe with complementary ribosomal RNA released from the target organism to form a stable DNA:RNA hybrid. These acid nucleic based probes are specific for a limited number of Fungi, including Histoplasma capsulatum, Blastomyces dermatitidis, and Cryptococcus neoformans. Other biological molecular test, such as PCR, allows the detection of fungi directly from clinical specimens and, in some instances, permits their quantification, too.

Unfortunately, a lack of test standardization and limited validation data for many fungal nucleic acid tests has hampered its implementation in clinical practice. The combination of real time PCR with a variety of testing platforms and probe-based detection systems (eg, molecular beacons, and fluorescent resonance energy transfer [FRET] hybridization probes) have also reduced the contamination due the post amplification and the time of the entire procedure [19-22].
PARASITES

The morphological identification of parasites is based on the use of conventional methods, such as optical microscopy. Leishmania and Toxoplasma gondii are frequently found in lymph nodal lesions due to infectious diseases. Leishmania is identifiable with Papanicolaou and Giemsa staining, Toxoplasma with Giemsa.

As for the ancillary techniques, molecular biology is applied to diagnose parasites structures; several molecular tests to detect parasites have been developed, increasing progressively their specificity and sensitivity.

Some examples of diagnostic techniques to establish parasite infections are: PCR, RT-PCR and restriction fragment length polymorphism (RFLP). PCR method allows the enzymatic amplification of DNA in vitro from small amounts of material. This aspect is particularly relevant in parasitology field because it is frequently impossible to obtain or isolate a sufficient amount of material from parasites for conventional analysis. Several PCR assays have also been developed for the direct detection of Leishmania in clinical samples.

Quantitative real-time PCR has been used to detect pathogens, the expression and regulation of genes, quantifying parasitic nucleic acids, too. Lin et al. [23] developed a RT-PCR to investigate Toxoplasma gondii.

This method is a rapid, sensitive, and quantitative technique to detect this parasite in clinical samples.

According to the authors, this test is particularly useful in the diagnosis of T. gondii in AIDS patients who generally do not generate increased immunoglobulin M (IgM) or immunoglobulin G (IgG) levels. Costa et al. [24] proposed a method to identify T. gondii by RT-PCR that can also be useful in monitoring treatment efficacy. Various aspects of leishmaniosises - diagnosis, animal models, drug efficacy and vectorial capacity - have been investigated using RT-PCR [25]. PCR methods have also been combined with RFLP to genotype organisms.

RFLP is one of the most commonly used molecular methods for diagnosis of species and genotypes of parasites such as Toxoplasma gondii.

This reaction is based on the use of digestion of the PCR products by restriction enzymes or endonucleases. DNA is cleaved into fragments of specific sizes, that can be visualized on agarose or polyacrylamide gel. Although the high costs of these techniques, these tests are gradually spreading in clinical diagnosis, treatment monitoring, and epidemiological studies of parasitic diseases.

MYCOBACTERIA

The resurgence of mycobacterial infections is due to different factors including the increasing of immunodeficiency syndromes. Infections by Mycobacterium tuberculosis and other mycobacteria are represented by pulmonary infections and lymphadenopathies. In many instances, when patients are immunocompromised or has already had previous mycobacterial infections, additional specimens should be obtained for culture and special stains. Diagnostic material is usually obtained for culture or fluorescence microscopy, PCR can be used for the rapid detection and identification of M. tuberculosis [19-22].

VIRUSES

Cytological preparations of FNC samples obtained from lymph nodes affected by viral infections do not allow the detection of corresponding virus whereas cytological features may show the viral cytopathic effects on infected cells. Cytomegalovirus (CMV) is an example of specific cytopathic modifications because CMV may show both nuclear and cytoplasmic inclusions that can be identified on both Papanicolaou and Diff Quik (DQ) stained smears. Nuclear inclusions are best visualized with Papanicolaou stain whereas cytoplasmic inclusions are bright magenta stained with DQ.

The diagnosis of viral infection has been hampered for long time because of high costs, time consuming and difficult laboratory systems used instead PCR technology has dramatically increased the possibilities of detection of viruses. Various detection methods, based on amplification systems, have been developed, such as the Hybrid Capture technique, a diagnostic in vitro test approved by Food and Drug Administration.

This system uses RNA probes that label target sequences of DNA forming hybrid complex RNA/DNA. The complex is captured on a solid phase by specific antibodies. Hybrid Capture permits the detection of some viruses, including Cytomegalovirus [19-22]. In conclusion, the
combination of FNC morphological data with molecular biology analysis represents a high efficient, minimally invasive and cost-effective procedure, mainly in cases in which an infectious process is expected.

Keywords: fine needle cytology, lymph node, infectious disease, ancillary techniques, molecular biology.

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SUMMARY

Fine needle cytology (FNC) represents a valid tool in the diagnosis of lymphadenopathies. When rapid on site evaluation (ROSE) is performed the method overcomes the problems related to the adequacy and allows to store residual material for ancillary techniques future. Cytological data, obtained by FNC may be supported and integrated by ancillary techniques namely molecular biology. The detection of specific microorganisms based on nucleic-acid technologies is the fundamental principle of molecular techniques, allowing a rapid diagnosis, an immediate treatment with minimal invasion and costs for the patient. Molecular procedures are also characterized by high levels of specificity and sensitivity. In conclusion, morphological diagnosis of infectious diseases performed on FNC samples can be enhanced by molecular analysis data.

REFERENCES