Fine needle cytology, infectious diseases and non-Hodgkin lymphoma

Citologia per ago sottile, malattie infettive e linfomi non-Hodgkin

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■ BACKGROUND

It has been estimated that about 25% of all human malignancies are caused by infectious organisms. Focusing on lymphomas and hematologic malignancies, several organisms have been implicated in disease etiology [1-7]. The association between some infections and non-Hodgkin lymphomas (NHL) is well known, along with other established risk factors such as hereditary immunodeficiency disorders, acquired states of immunosuppression, some autoimmune disorders and a positive family history of lymphoproliferative malignancies. Three main mechanisms have been implicated in viral-induced pathogenesis of NHL: in the first mechanism, viruses can directly infect lymphocytes and act as promoters of cell proliferation.

In the second mechanism, depletion of CD4+ T lymphocytes caused by human immunodeficiency virus (HIV) infection determines elevated risk of high-grade B-cell NHL. In the third one, some infections increase NHL risk by inducing chronic immune stimulation and persistent activation of lymphocytes [8]. Long standing infectious diseases may represent the background for lymphproliferative processes and clinical evaluation of the corresponding lymph node enlargement can be blurred by the overlapping of different pathologies. Surgical excision and histological control still represent the gold standard in the diagnosis of lymphadenopaties, but Fine Needle Cytology (FNC) combined with ancillary techniques when needed, can also produce correct diagnoses and may be conveniently used as first line diagnostic procedure [9-21].

FNC in the diagnosis of non-Hodgkin lymphoma
In case of suspected NHL, the first step of lymph node FNC is to assess the malignancy of the process. This may be obtained in some cases by cytological features alone, mainly in cases of high grade lymphoma (Figure 1). In all other cases, the cytological diagnosis has to be supported by the demonstration of the B or T monoclonality, generally obtained by flow cytometry (FC), by the demonstration of specific chro-
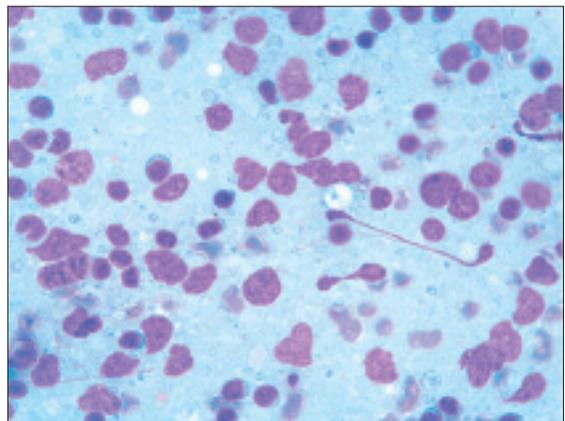

Figure 1 - FNC of a high grade lymphoma, lymphoid cells are large with evident nuclear atypia indicative of malignancy (Diff Quik stain 430X).
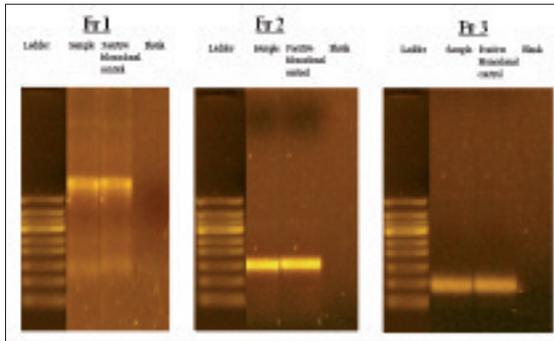
Figure 2 - PCR amplification of IGH frameworks regions showing 2 monoclonal bands in Fr2 and in Fr3.

mosomal translocations by Fluorescence In Situ Hybridization (FISH) or by PCR amplification of IGH frameworks regions (Figure 2). When a defined diagnosis of NHL has been assessed, a possible classification may be attempted combining cytological features and phenotypic patterns using immunocytochemistry (ICC) and/or FC.

Many laboratories have adopted FNC/FC and FNC/FISH for the diagnosis of lymph nodal and extra-nodal lymphoid disorders [22-25]. As for the clinical value of FNC diagnoses, many institutions accept cytological diagnoses of NHL without histological controls, in cases of reactive processes, metastases, NHL relapses, and in primary cases in specific clinical condition, whereas histological confirmation is necessary for primary diagnoses and in all cases where cytological, clinical and instrumental findings appear contradictory.

■ CYTOLOGICAL FINDINGS

T-cell lymphoma, being or not virus type 1 (HTLV-1) related, may be quite difficult to diagnose on histological as well as on cytological samples. Corresponding smears may have a polymorphous or monomorphous pattern with the presence of atypical cells often showing nuclear abnormalities. The diagnosis depends on cytological atypical cells as well as on the demonstration of the T phenotype of the corresponding atypical cells and their clonality by the loss of T- specific antigens and or the T-cell receptor rearrangement by molecular procedures. The Epstein-Barr virus (EBV) associated Burkitt's lymphoma (BL), in both endemic and sporadic presentations, shows the t(8;14)

translocation with activation of the c-*myc* oncogene and this translocation can be detected by FISH on cytological material. FNC shows a cell population composed of undifferentiated medium sized lymphocytes with dispersed chromatin and one or more small basophilic nucleoli; the cytoplasm is basophilic and sometimes vacuolated. Immature nuclei are fragile, resulting in frequent nuclear crushes in the smear; a high mitotic index is constantly present and scattered macrophages with engulfed cytoplasm may give the peculiar "starry sky" pattern to the smear.

FC will show a highest expression of CD10 and CD19 with or without evidences of light chain expression and FISH can demonstrate the specific translocation t8-14. EBV is also related to Hodgkin lymphoma (HL), post-transplantation proliferative disorders, extranodal NK-/T-cell lymphoma (nasal type) and B cell NHL arising in patients infected with HIV. These patients have markedly elevated risk of three NHL subtypes: central nervous system NHL, BL, diffuse large B-cell lymphoma (DLBCL) [8]. In DLBCL, cytological findings show a cell population composed of large lymphoid cells with irregular nuclei, one or more large nucleoli and a wider rim of pale cytoplasm. Morphological variants exist in which nuclei may be slightly irregular in shape or cleaved and have dense chromatin and indistinct nucleoli. All the cytological diagnoses have to be sustained by appropriate phenotypical and/or molecular procedures.

Persistent HCV infection is associated with a range of immune-related conditions, including essential mixed cryoglobulinemia, and low-grade lymphoproliferative disorder that can progress to NHL [26]. It is not clear whether a specific subtype of NHL is associated with the infection or, conversely, that an increased risk exists for all NHL subtypes. Mucosa-associated lymphoid tissue (MALT) NHLs arise in aggregates of lymphocytes associated with sites of chronic inflammation. There is growing evidence linking chronic gastritis caused by *Helicobacter pylori* (HP) to MALT NHL at those sites. Prevalence of HP infection is higher among individuals with gastric MALT NHL than among controls, and HP antibacterial therapy can lead to regression and remission of the associated tumor in many cases [27].

In these cases, cytological specimens can be obtained by endoscopic ultrasound guided- FNC of satellite lymphnodes.

CONCLUSIONS

Fine Needle Cytology in an effective tool in the diagnosis and classification of NHL, provided that it is coupled with ancillary techniques and performed by expert cytopathologists.
Histological diagnosis is still gold standard for primary diagnosis as well as for all cases in which FNC/FC does not succeed.
FNC may simplify the whole diagnostic procedure, avoiding surgical biopsies and speeding up therapeutic procedures in relapse NHL and in selected primary NHL cases.

Keywords: lymph node, fine needle cytology, infectious diseases, lymphoma.

SUMMARY

The association between some infections and non-Hodgkin lymphomas (NHL) is well known. Human T-cell leukemia/lymphoma virus type 1 (HTLV-1) was the first oncogenic human retrovirus to be discovered and has been found to be associated with adult T-cell leukemia/lymphoma (ATLL). Epstein-Barr virus (EBV) has consistently been linked to both endemic and sporadic Burkitt's lymphoma (BL), as well as to Hodgkin lymphoma (HL), post-transplantation proliferative disorders, extra-nodal NK-/T-cell lymphoma (nasal type) and B-cell NHL arising in HIV patients; HCV infection is also associated to low-grade lymphoproliferative disorders that can progress to NHL. Bacterial infections have also been associated to NHL; chronic gastritis caused by *Helicobacter pylori* is responsible for mucosa-associated lymphoid tissue (MALT) NHL and high prevalence of Chlamydia psittaci infections has been reported in ocular adnexal lymphomas. In both these conditions, infection may contribute to the development of lymphomas, as proven by the clinical responses eradicating antibiotic therapies.
Histological diagnosis coupled with immunohistochemical and molecular procedures are needed for a definitive diagnosis, but Fine Needle Cytology (FNC) combined with ancillary techniques can also produce correct diagnoses in most cases. In patients suffering from NHL, FNC also plays an important role in differential diagnosis between relapse of primary disease and reactive lymph nodes enlargement. This review explores the role of FNC in the diagnosis and classification of NHL trying to highlight possibilities and the limitations of the technique.

RIASSUNTO

L'associazione tra agenti infettivi e l'insorgenza di linfomi non Hodgkin (NHL) è nota. Il primo virus identificato come responsabile di una malattia linfoproliferativa è stato il virus umano linfotropo per le cellule T (HTLV-1) associato alla leucemia/linfoma a cellule T dell'adulto. L'infezione da virus di Epstein-Barr è strettamente associata al linfoma di Burkitt, sia endemico che sporadico, ma anche al linfoma di Hodgkin, ai disordini linfoproliferativi post trapianto, al linfoma extranodale a cellule NK/T e ai NHL B cellulari che insorgono in pazienti affetti da HIV. Anche l'infezione dal virus dell'epatite C può causare linfomi di basso grado. Tra i batteri implicati nella linfomagenesi, l'Helicobacter pylori è coinvolto dello sviluppo di NHL del tessuto linfoide associato alle mucose (MALT) ed infezioni da Chlamydia psittaci sono spesso associate a linfomi degli annessi oculari. In queste patologie la valutazione istologica è la base diagnostica delle corrispondenti linfoadenopatie, tuttavia la citologia per ago sottile (FNC), associata a tecniche ancillari, può fornire una corretta diagnosi e classificazione pre-operatoria nella maggior parte dei NHL contribuendo potenzialmente anche alle strategie terapeutiche. L'FNC può svolgere inoltre un ruolo anche nella diagnosi differenziale tra recidiva di linfoma e linfadenopatie reattive in pazienti con storia di NHL. In questo studio sono descritti gli aspetti citologici osservabili in linfonodi affetti dai principali NHL associati ad agenti infettivi batterici e virali, valutando le possibilità e i limiti del FNC nella loro diagnosi e classificazione.

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