Le Infezioni in Medicina, Suppl. 3, 34-38, 2012

Lymph node fine needle cytology, Epstein Barr virus infection and Hodgkin Lymphoma

Citologia per ago sottile dei linfonodi, infezione da Epstein Barr virus e linfomi di Hodgkin

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INTRODUCTION

Epstein-Barr virus (EBV) is one of the most common viruses occurring in humans. It is associated with a wide spectrum of benign and malignant diseases, such as infectious mononucleosis, and it is linked to 1% of tumours. It is related to the development of several neoplasms, including epithelial, mesenchymal and lymphoproliferative neoplasm. These latter include Hodgkin lymphoma (HL) and B or T cell non-Hodgkin lymphoma (NHL). Fine Needle Cytology (FNC) is extensively used in the first diagnosis of any lymph-nodal enlargement, including reactive lymphadenopathies, and hematological malignancies and lymphomas [1-23], therefore cytologists are likely to encounter EBV-associated malignancies in cytology material, namely Hodgkin lymphoma.

EBV and oncogenesis

EBV is a double-strand DNA virus of the herpes family also known as human herpesvirus 4 (HHV-4); it infects more than 95% of the world's population, showing a strong tropism for epithelial cells and lymphocytes (B and T cells). It has both a latent phase of infection and a lytic phase. Primary infection may cause infectious mononucleosis, being most infections initially asymptomatic. Latent infection in cells is characterized by the expression of latent membrane proteins (LMP) 1 and 2, by EBV nuclear antigens (EBNAs) and by EBV encoded RNAs (EBERs). EBERs are highly transcribed in latent infections allowing the survival of the viral genome and the risk of neoplastic transformation. In an immunocompetent host, EBV infection is kept under control, which is why the vast majority of the population never develops EBV-associated tumours. However, unlike other herpes-viruses, EBV does not evoke a viral cytopathic effect and clinical manifestations may vary on the basis of the patient's immune status.

Hodgkin lymphoma: different entities and subtypes

HL is one of the most common lymphomas in the developed world, with an incidence of approximately 3 per 100,000 person-years with a bimodal incidence affecting both young people and adults. HL clinical presentation includes B-symptoms (fever, night sweats and weight loss), lymph nodes enlargement and extranodal disease [24, 25]. HL comprises two entities, nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and classical Hodgkin lymphoma (cHL). A striking feature of both entities is the scanty presence of malignant cells such Hodgkin cells (HC) and Reed-Sternberg (RS) cells in the involved tissue, accounting for only around 1% of the tumour

mass. The remaining neoplasm comprises a reactive cellular infiltrate with an admixture of different cell types. Classic HL accounts for 95% cases of HL and is further divided into 4 subtypes on the basis of the HRS cells type and the composition of the cellular infiltrate in the background. Approximately one-third of cases of cHL in the developed world is associated with EBV.

EBV and Hodgkin lymphoma

In EBV-associated HL, the virus is detected in all the neoplastic cells and HRS cells express EBV proteins including EBNA-1, LMP-1, LMP-2 antigens; these findings further confirm that viral infections have a causative role in neoplastic transformation. EBV plays a role in the survival of neoplastic cells through dysregulation of several molecular pathways and transcription factors such as the nuclear factor (NF)-kB, which plays a key role in numerous cellular responses, including inflammatory responses and cell fate decisions. NF-kB activation is normally transient and highly controlled; however, NF-kB is constitutively active in HRS cells and epidemiological data also support a role for EBV in HL pathogenesis. There is a variability in the percentage of EBV involved cases between racial groups: the proportion of EBV involvement is almost 100% in Hispanic cHL patients, being much lower in Caucasians (20%) and intermediate in Asians. EBV association with cHL is related to age, being the strongest in children and the elderly. In addition, male sex and the mixed cellularity histological subtype are associated with EBV+ cHL worldwide [26]. These data suggest that control of EBV infection is related to the risk of developing EBV-associated HL.

FNC of Hodgkin lymphoma

FNC smears from HL lymph nodes may be hypo- or hyper-cellular on the basis of associated sclerosis; cHL is characterized by a minority of RS cells and their variants in an inflammatory background (Figure 1). Non-neoplastic inflammatory cells consist of small lymphocytes, plasma cells, neutrophils, eosinophils and macrophages, in a variable number. In EBV-associated cases, macrophages may show prominent epithelioid features even with a granulomatous pattern; necrosis is seldom observed. Classic RS cells are bi-nucleated cell and often the two nuclei are mirror images of each other or multinucleated with pale, finely granular

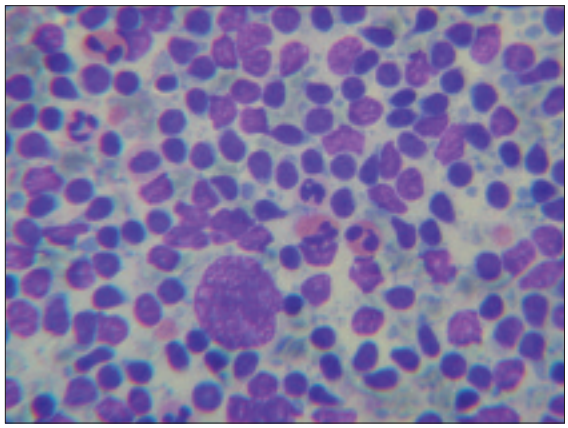

Figure 1 - Fine needle cytology smear of Hodgkin lymphoma: mononucleated atypical cell in a polymorphous background; note the numerous eosinophils (Diff Quik stain 430 X).

chromatin with huge nucleoli and a moderate amount of cytoplasm (Figure 2) and are positive for CD15 and CD30. Not all histological subtypes of HL may be reliably identified by FNC. As reported in the literature, HL cytological diagnosis may be hampered by different factors such as specific histological subtype, shortage of diagnostic cells and hidden or masked diagnostic cells. Indeed, in nodular sclerosis, which represents the most common histological subtype of HL, fibrosis and random distribution of diagnostic RS cells may hamper the cytological integrity, harvest and examination of diagnostic cells. RS cells may also be scanty and the presence in a cytological sample of variants such as ''atypical mononu-

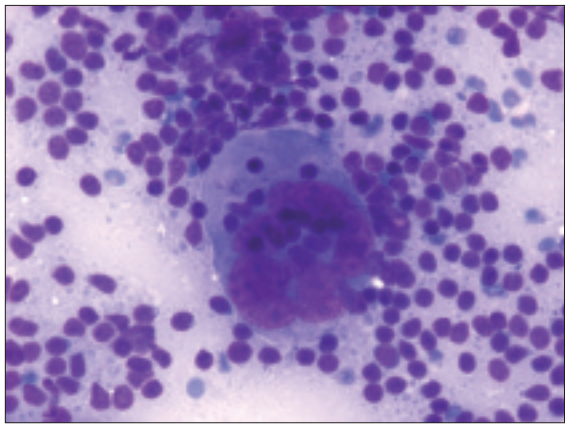

Figure 2 - Classic Reed-Sternberg cell in a lymphoid back ground as appears in a lymph node FNC of Hodgkin lymphoma (Diff Quik stain 430 X).

clear nucleolated'' or ''hyperlobated'' may suggest HL, but might not be sufficient for a definitive diagnosis of HL. In ordinary cases, RS cells and their variants are generally interspersed in a reactive background that may hide diagnostic cells and lead to false-negatives. Conversely atypical cells, mimicking RS cells may be observed in reactive lymph-nodes or NHL such as anaplastic ''K1'' lymphoma, melanoma and some undifferentiated carcinomas. All these difficulties may probably cause the extreme variability of sensitivity in the different experiences reported in the cytological diagnosis of HL [27]. Therefore, primary diagnosis of HL must be confirmed by subsequent histological examination.

EBV infection in Hodgkin lymphoma: ancillary studies

Various methods can demonstrate the presence of EBV infection. In the host, these include serological tests showing "heterophile antibody", enzyme-linked immunosorbent assays (ELISA) and EBV viral-load assays which help distinguish a healthy carrier from one in the disease state. EBV viral-load assays have been used in the management of post-transplant lymphoproliferative disorder and nasopharyngeal carcinoma, whereby EBV DNA levels in plasma or serum determine response to therapy; high levels prior to therapy carry a worse prognosis and test recurrence. Within tumours, EBV infection is confirmed with commercially existing antibodies against EBNAs, detected by immunofluorescence, and LMP-1 by immunohistochemical staining, RNA in situ hybridization to detect EBERs and/or molecular studies such as Southern blot hybridization and polymerase chain reaction (PCR). EBER in situ hybridization is perhaps the best test for detecting and localizing latent EBV in tissue and cytology samples [25, 28].

False-positive EBER interpretations may also occur as a result of confusion regarding the latent infection of background lymphocytes instead of lymphoma cells, nonspecific staining or cross-reactivity with mucin, yeast or plant materials. False-negative results may occur with RNA degradation, therefore an appropriate RNA control should be examined when interpreting an EBER in situ hybridization test [25].

CONCLUSIONS

FNC is a useful tool for the diagnosis of HL. Knowledge of the patient's EBV status and immune competence combined with the cytomorphology and results of ancillary studies may be useful in the management of patients and FNC material may contribute to this purpose.

Keywords: Lymph Node, Fine Needle Cytology, EBV Infection, Hodgkin Lymphoma.

Conflict of interest disclosure: The authors declare that the article has not been sponsored, that no financial support has been given and finally that there is no conflict of interest.

SUMMARY

Epstein-Barr virus (EBV) is a double-strand DNA virus of the herpes family; it is one of the most common human viruses and it is associated with a wide spectrum of benign and malignant conditions. EBV is related to the development of several neoplasms, globally 1% of tumours, including lymphoproliferative, epithelial and mesenchymal neoplasm. Lymphoproliferative disorders include Hodgkin lymphoma (HL) and B and T cell non-Hodgkin lymphoma. HL is one of the most common lymphoma in the developed world, affecting both young people and adults. HL pathogenesis is complex and includes various and partially unknown mechanisms. EBV has been detected in some HL neoplastic cells and expresses genes with a potential oncogenic function, therefore many studies suggest that viral infections have a causative role in neoplastic transformation. Fine Needle Cytology (FNC) is extensively used in the first diagnosis of any lymph-nodal enlargement, including reactive lymphadenopathies and lymphoproliferative processes; therefore cytopathologists are likely to encounter EBV-associated malignancies in cytology samples, mainly HL, which is one of the most common lymphoma. This study focuses on the cytological features and ancillary studies required to diagnose EBV-related HL.

RIASSUNTO

Epstein-Barr virus (EBV) è un virus a DNA a doppia elica, appartenente alla famiglia degli Herpesvirus e rappresenta uno dei virus più diffusi. EBV è associato con un ampio spettro di patologie, sia benigne che maligne. EBV è inoltre correlato allo sviluppo di differenti neoplasie, complessivamente 1% di tutti i tumori, comprendenti neoplasie linfoproliferative, epiteliali e mesenchimali. I processi linfoproliferativi comprendono il linfoma di Hodgkin (LH) e linfomi non-Hodgkin a cellule B e T. Il LH è uno dei linfomi più comuni, che colpisce sia giovani che adulti. La sua patogenesi è complessa e comprende vari meccanismi in parte ancora sconosciuti. L'EBV è presente nelle cellule neoplastiche del LH di una percentuale di casi ed esprime geni con una potenziale attività oncogena, pertanto si ritiene che l'infezione virale possa avere un ruolo patogenetico nella trasformazione neoplastica. La citologia per ago sottile (FNC) è ampiamente utilizzata nella diagnosi iniziale delle linfoadenopatie comprendenti sia processi reattivi che linfomi. Pertanto i citopatologi possono dover affrontare neoplasie EBV-correlate in campioni citologici ed in particolare il LH. In questo studio sono riportati gli aspetti citomorfologici e le tecniche ancillari necessarie per la diagnosi di LH.

REFERENCES

[1] Messina G., Giaccone L., Festuccia M., et al. Multicenter experience using total lymphoid irradiation and antithymocyte globulin as conditioning for allografting in hematological malignancies. *Biol. Blood Marrow Transplant.* 18, 1600-1607, 2012.

[2] Risitano A.M., Serio B., Selleri C., Rotoli B. Valganciclovir as CMV reactivation prophylaxis in patients receiving alemtuzumab for marrow failure syndromes. *Ann. Hematol.* 88, 1261-1262, 2009.

[3] Risitano A.M., Perna F., Selleri C. Achievements and limitations of complement inhibition by eculizumab in paroxysmal nocturnal hemoglobinuria: the role of complement component 3. *Mini Rev. Med. Chem.* 11, 528-535, 2011.

[4] Selleri C., Serio B., Risitano A.M. Novel immunosuppressive strategies for bone marrow failure syndromes: a focus on alemtuzumab. *Mini Rev. Med. Chem.* 11, 536-543, 2011.

[5] Risitano A.M., Selleri C., Serio B., et al. Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anaemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA. *Br. J. Haematol.* 148, 791-796, 2010.

[6] Cozzolino I., Vigliar E., Sosa Fernandez L.V., et al. Non lymphomatous clonal B-cell population in enlarged lymph nodes in acquired immunodeficiency syndrome. *Infez. Med.* 20, 35-42, 2012.

[7] Petruzziello F., Zeppa P., Ciancia G., et al. Cytological and histological detection of amyloid deposits in bone marrow of patients affected by multiple myeloma. *Leuk. Lymphoma.* 52: 2304-2307. 2011.

[8] Cozzolino I., Zeppa R., Zeppa P. Lymph nodal Merkel cell carcinoma: primary tumor or metastasis from unknown primary site? *J. Cutan. Pathol.* 10, 836-837, 2011.

[9] D'Antonio A., Paolella G., Zeppa P. Rapidly growing intraparotid mass in a young child. *J. Craniofac. Surg*. 4, 305-306, 2012.

[10] Zeppa P., Sosa Fernandez L.V., Cozzolino I., et al. Immunoglobulin heavy-chain fluorescence in situ hybridization-chromogenic in situ hybridization DNA probe split signal in the clonality assessment of lymphoproliferative processes on cytological samples. *Cancer Cytopathol*. 2012 (in press).

[11] Bellevicine C., Malapelle U., Iaccarino A., et al. Foamy gland pancreatic ductal adenocarcinoma diagnosed on EUS-FNA: a histochemical, immunohistochemical, and molecular report. *Diagn. Cytopathol*. 2012 (in press).

[12] Stanzione B., Cozzolino I., Arpino G., Vigliar E., Virginia S.F., Zeppa P. Multiple metachronus proliferative fasciitis occurring in different anatomic regions: a case report and review of the literature. *Pathol. Res. Pract*. 2, 126-130, 2012.

[13] Zeppa P., Cozzolino I., Peluso A.L., et al. Cytologic, flow cytometry, and molecular assessment of lymphoid infiltrate in fine-needle cytology samples of Hashimoto thyroiditis. *Cancer*. 3, 174-184, 2009.

[14] Troncone G., Guerriero E., Pallante P., et al. UbcH10 expression in human lymphomas. *Histopathology* 54, 6, 731-740, 2009.

[15] Malapelle U., Bellevicine C., Zeppa P., Palombini L., Troncone G. Cytology-based gene mutation tests to predict response to anti-epidermal growth factor receptor therapy: a review. *Diagn. Cytopathol.* 9, 703-710, 2011.

[16] Zeppa P., Barra E., Napolitano V., et al. Impact of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) in lymph nodal and mediastinal lesions: a multicenter experience. *Diagn. Cytopathol*. 10, 723-729, 2011.

[17] Zeppa P. Haematocytopathology: why? *Cytopathology*. 2, 73-75, 2012.

[18] Zeppa P., Vigliar E., Cozzolino I., et al. Fine needle aspiration cytology and flow cytometry immunophenotyping of non-Hodgkin lymphoma: can we do better? *Cytopathology*. 5, 300-310, 2010.

[19] Zeppa P., Varone V., Cozzolino I., Salvatore D, Vetrani A., Palombini L. Fine needle cytology and

flow cytometry of ectopic cervical thymoma: a case report. *Acta Cytol*. 5, 998-1002, 2010.
[20] Vigliar E., Bellevicine C., Cozzolino I., Zeppa P. Histological and fine needle aspiration cytological features of Hashimoto thyroditis-associated 'angiomatoid' papillary thyroid carcinoma. *Cytopathology*. 2012 (in press).
[21] Cozzolino I., Nappa S., Picardi M., et al. P. Clonal B-cell population in a reactive lymph node in acquired immunodeficiency syndrome. *Diagn. Cytopathol.* 12, 910-914, 2009.
[22] Vigliar E., Cozzolino I., Fernandez L.V., et al. Fine-needle cytology and flow cytometry assessment of reactive and lymphoproliferative processes of the breast. *Acta Cytol.* 2, 130-138, 2012.
[23] D'Antonio A., Baldi C., Memoli D., Caleo A., Rosamilio R., Zeppa P. Fine needle aspiration biopsy of intraparotid spindle cell lipoma: A case report. *Diagn. Cytopathol*. 2011 (in press).
[24] Farrell K., Jarrett R.F. The molecular pathogenesis of Hodgkin lymphoma. *Histopathology* 58, 15-25, 2011.
[25] Michelow P., Wright C., Pantanowitz L. A review of the cytomorphology of Epstein-Barr virus-associated malignancies. *Acta Cytologica* 56, 1-14, 2012.
[26] Huang X., Nolte I., Gao Z., et al. Epidemiology of classical Hodgkin lymphoma and its association with Epstein Barr virus in Northern China. *PLoS One* 6, e21152, 2011.
[27] Zeppa P., Picardi M., Cozzolino I., et al. Fine-needle aspiration cytology in the follow-up of Hodgkin lymphoma. *Diagn. Cytopathol*. 467-472, 2008.
[28] Hecht J., Cibas E., Kutok J. Fine-needle aspiration cytology of lymphoproliferative disorders in the immunosuppressed patient: the diagnostic utility of is situ hybridization for Epstein-Barr virus. *Diagn. Cytopathol* 26, 360-365, 2002.