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Non lymphomatous clonal B-Cell populations in enlarged lymph nodes in acquired immunodeficiency syndrome

Popolazioni linfocitarie B-cellulari clonali, non linfomatose, in linfoadenopatie in corso di sindrome da immunodeficienza acquisita

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INTRODUCTION

Clonal expansion of monotypic B or T cell populations is generally considered the hallmark of lymphomatous process and the detection of clonality in B or T-lymphocytes a marker of malignancy in lymphoid proliferations. Nonetheless some studies have detected clonal B-cell populations by molecular techniques and/or by light chain restriction in lymph nodal and extra nodal, non-lymphomatous, B-cells proliferations [1-14].

These studies have mainly highlighted some bacterial, viral, or autoimmune pathological processes such as Helicobacter pylori chronic gastritis, HCV hepatitis, EBV infections, Hashimoto thyroiditis, or Sjogren's syndrome as the most frequent non-lymphomatous clinical settings in which clonal B-cell populations may be detected [1-12].

More recently, the same phenomenon has also been reported in HIV-related acquired immunodeficiency syndrome [13, 14]. Fine needle cytology (FNC) is an established technique in the diagnosis of lymph nodes enlargement and lymphproliferative processes [15-21]. When lymph node FNC is coupled with flow cytometry (FC), fuorescence in situ hybridization (FISH) or molecular techniques in the analysis of the obtained cells, the procedure offers high diagnostic values of sensitivity and specificity [22-26]. In this study we report three consecutive cases of clonal B-cell population, detected in reactive lymph nodes, occurring in HIV positive patients. One of the patients suffered from acquired immunodeficiency syndrome (AIDS) and had also suffered from a follicular non-Hodgkin lymphoma (FL); preliminary data of this case have been described in a previous report [21].

PATIENTS AND METHODS

Over a period of three years from January 2007 to December 2011, FNAC/FC was used to analyse 572 cases of benign reactive hyperplasia (BRH), NHL and NHL relapse (rNHL) in 478 lymph nodes and 94 extranodal lesions. The procedure were performed by cytopathologists either on palpable mass either under ultrasound or CT control.

Forty-two cases concerned HIV patients in which FNC was used to prepare conventional smears and cell suspensions for FC. Among these patients three consecutive cases showing light chain restriction at FC analysis and revealed to be reactive at the following histological control in the first two cases and at the clinical follow up were retrieved and retrospectively studied. The first patient was a 40-year-old female, HIV positive, stage C, since 3 years,

complained of a right-cervical swelling. Two years before, the patient had been diagnosed with a follicular B-cell non-Hodgkin lymphoma (FL); the patient had been treated with four cycles of multiagent chemotherapy plus rituximab, the last cycle being administered 10 months before the lymph node enlargement. The second and third patients were two males, 31 and 43 yrs old respectively.
The clinical histories of these two latter patients were quite similar being both drug abusers and HIV positive since 5 and 9 years respectively; both with a history of different recurrent infectious processes.
All the investigated lymph nodes were oval, ranging between 30 and 20 mm in diameter showing a preserved hilus. In all the cases FC phenotypization on cell suspension was performed using the following fluoresceinated antibodies: CD3, CD5, CD19, CD23, FMC7, CD10, kappa, and lambda light chain and a three-color analysis technique on a Becton Dickinson (San José, CA) FACS scan, as previously described [15-21]. In the first two cases corresponding lymph nodes were excised and diagnosed as florid follicular hyperplasia; a molecular analysis was also performed on the surgical sample of the first case to evaluate the amplified variable diversity joining (VDJ) region of the heavy chain Ig genes (IgH). The third patient refused the surgical excision and was clinically controlled.

RESULTS

Cytological smears were quite similar in all the cases being highly cellular and showing a lymphoid polymorphous cell population. Cells were mainly small and large lymphocytes; the small ones were round with compact chromatin without nucleoli, whereas the larger lymphoid cells were round, centroblast-like, showing dispersed chromatin and two or three large, peripheral located nucleoli (Figure 1); in some areas the smears in cases 1 and 3, the large cells represented a large part of the whole cell population. Macrophages with large debris-engulfed cytoplasm, as well as some mitotic figures were present in all the cases. FC was performed by analyzing thousand cells per tube acquired and analyzed, as events.
Cells with very low forward scatter, which could include debris and degenerated cells, were excluded from the analysis by forward-versus side-scatter gating. CD19 versus side-scatter gating was used to limit the flow cytometric analysis of the B cells. In these three cases, FC analysis showed quite similar phenotypes that evidenced a reduced T cell component (CD3 and CD5 <10%), a large B cell component (CD19 and CD10 >40%) with CD10/19 >30% (Figure 2) and light chain restriction (kappa 2 cases) and lambda (1 case) (Figure 3). On the basis of cytological and FC results, a relapse from the former FL was diagnosed in the first patient and a suspect FL in the second one. Both were referred to the Hematology Department, where underwent to serological and radiological staging.

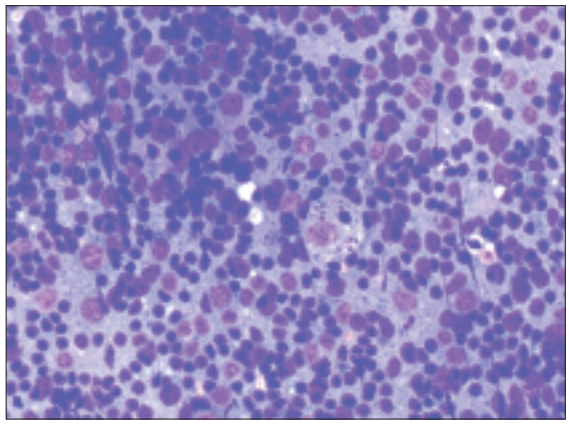

Figure 1 - Cytological features of case n. 2 showing large, nucleolated follicular centre cells intermingled with small lymphocytes and macrophages (Diff Quik stain 430 X).

The FDG-PET/CT scan showed positivity in the corresponding cervical areas whereas low serum LDH levels and a reduction of the lymph node sizes were also observed. Because of the cytological and FC data corresponding lymph nodes were excised and the histology revealed reactive hyperplasic lymph node with florid follicular patterns in both the cases.
The molecular analysis performed on the surgical sample of case 1, on the amplified variable diversity joining (VDJ) region of the heavy chain Ig genes (IgH), did not evidence any rearrangement.
As reported above, the third patient refused the surgical excision and was clinically controlled only and after two months the lymph node shrunk and almost disappeared. Clinical follow-up and periodical US and PET examinations did not evidence other regional lymph nodes in all the patients. All the patients are alive without sign of lymphoma.

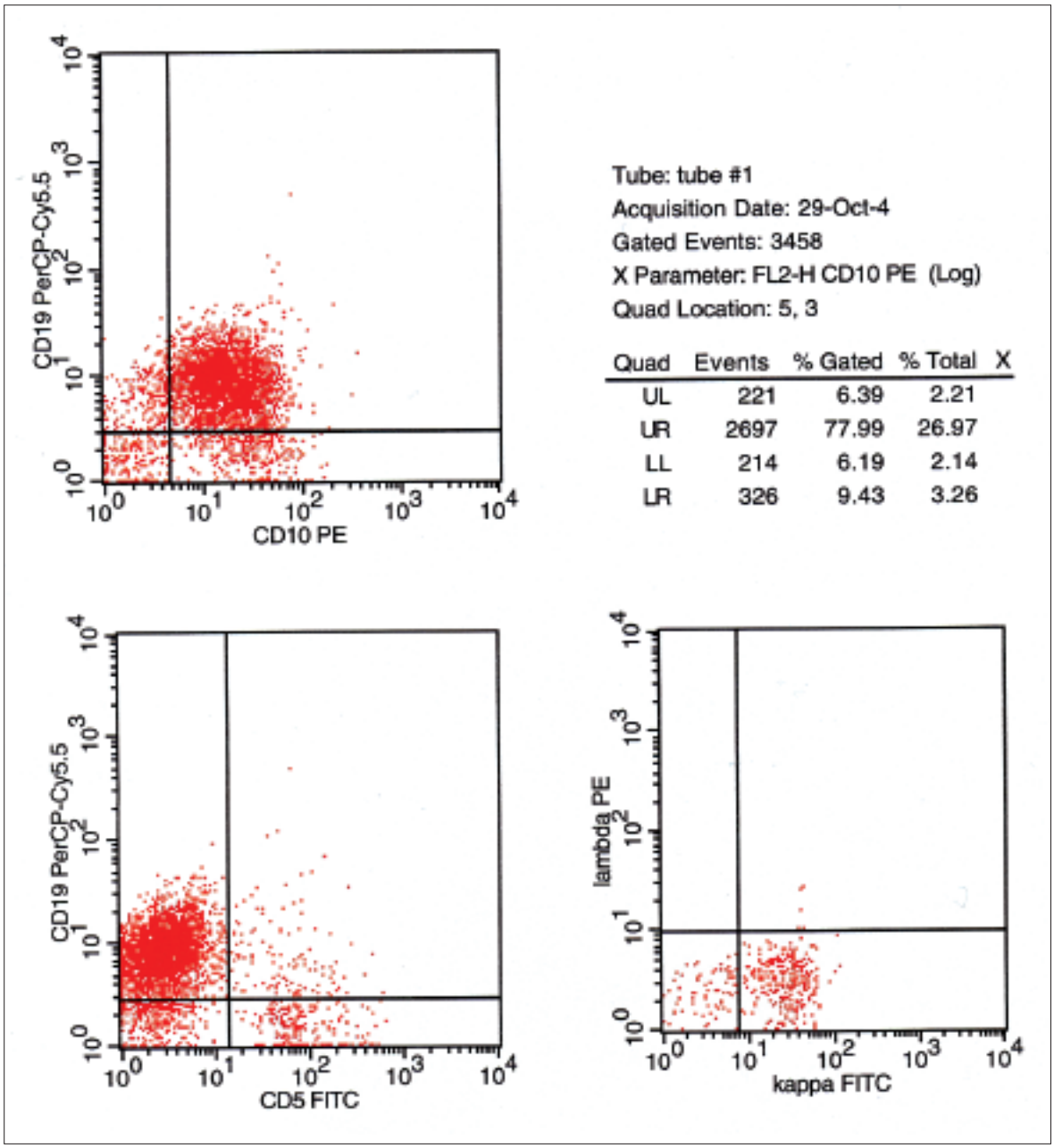

Figure 2 - Histogram of the same case showing al large CD19 positive, B-cell population in upper right quadrant and a small cluster of CD5 positive, T-cell population in lower right quadrant.

DISCUSSION

Several reports have highlighted the possible presence of clonal B-cell populations in non-lymphomatous processes. [1-14]. This phenomenon has been identified by molecular studies performed on mucosa-associated lymphoid cell populations [4-6, 14] or by the evaluation of light chain assessment of lymph nodal and non lymph-nodal cell populations by immunohistochemistry (IHH), immunocytochemistry (ICC), or FC [3, 7, 9, 10, 13].

This phenomenon seems involve less than 1% of all reactive lymph nodes [7] whereas in extra lymph nodal lymphoid cell populations, it seems to be more common [4, 6, 14]. In the cases reported in the literature [1-12], this clonality was identified by PCR or FC, in the latter with

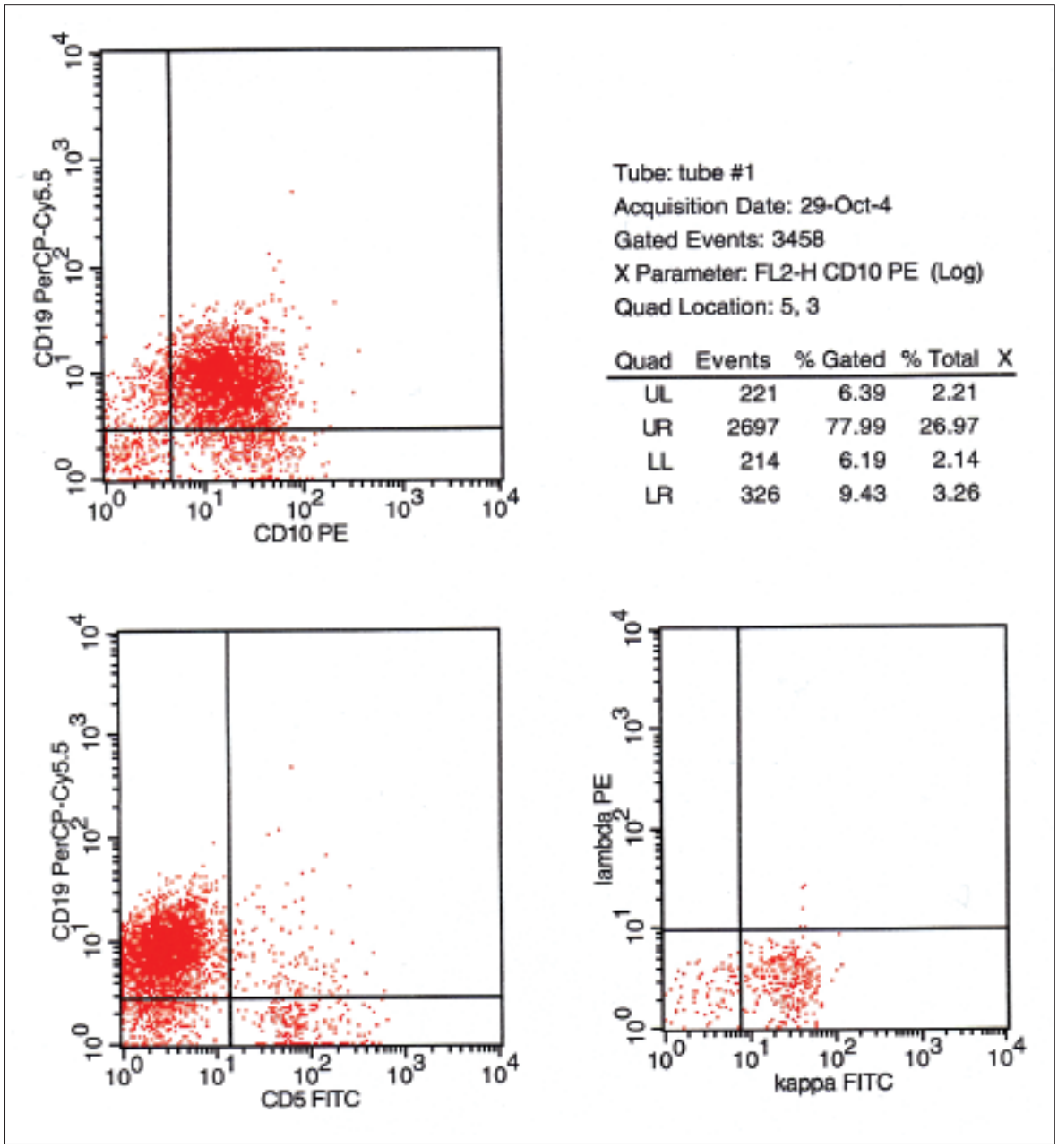

Figure 3 - Histogram of the same case showing a small monoclonal kappa light chain cluster in the lower right quadrant.

or without IgH rearrangement. Light chain restriction, detected by IHH, ICC, or FC is the epiphenomenon of clonal IgH rearrangement; therefore, when light chain restriction is detected in a quantitatively significant percentage of cells, IgH rearrangement should also be detected. Nonetheless, in the cases described in the literature this relationship was not constantly detected, especially in mucosa-associated lymphoid tissues of thyroid or salivary glands [6, 8, 13]; in addition, where detected, the IgH rearrangement was not present in a quantitatively consistent way.

In fact, Kussick et al. [7] found IgH gene rearrangements in three out of the six reactive lymph nodes with light chain restriction, and identified the corresponding rearrangement in a polyclonal background in two of the three

cases. In another study, Nam-Cha et al. [9] identified IgH rearrangement in half of their reactive lymph nodes.
This variability in IgH rearrangement detection might be related to the involvement of a small number of follicles; in fact, Nam-Cha et al. [9] detected IgH rearrangement in microdissected follicles in which light chain restriction was evidenced by IHH.
In all the other cases where a whole section of tissue was processed, follicles without rearrangement might represent the majority and mask the rearranged follicles. In our first case, DNA for PCR amplification of the IgH sequence was extracted from a whole section of paraffin embedded tissue, therefore a possible rearrangement in some follicles might have been blurred by the polyclonal background. To classify the phenotypical and molecular features of this phenomenon, different conditions with different modification of the genetic assessment have been described in the literature [1-14].
In some cases, only light chain restrictions have been reported, without IgH rearrangement [8,12,13]; most of these were found mainly in mucosa-associated lymphoid cell populations, as in HT, and where, in addition to light chain restriction, IgH rearrangement had been detected in a variable percentage of the corresponding tissue [7, 9, 11]. This is mainly the case of reactive lymph nodes in patients suffering from Castleman's disease, autoimmune or viral diseases (Herpes Virus 6, EBV, HIV).
In these conditions, neither translocation [14-18] nor Bcl-2 over-expression should be present. In fact, specific cases where, in addition to light chain restriction and IgH rearrangement, there is evidence of translocation 14-18 and the corresponding Bcl-2 over expression in some follicles selected by microdissection, have been considered as ''lymphoma in situ''15 and should not be considered as part of this phenomenon.
Therefore, the common hallmark of clonal, B-cell, non-lymphomatous processes involving mucosa-associated lymphoid cell populations and lymph nodes, is light chain restriction-often involving a small percentage of gated cells, in addition to IgH rearrangement of a limited number of follicles, lack of Bcl-2 over expression, and absence of 14-18 translocation [7-11]. Because of these specific features, the finding of clonal B-cell populations in non-lymphomatous processes has been considered an abnormal response to bacterial or viral antigen or autoimmune stimulations, and the corresponding light chain restrictions as the expression of somatic hyper mutation involving a variable percentage of the cells [2, 7, 11].
As for the relationships with lymphoma, as reported earlier, this phenomenon should not be considered an ''early'' or ''in situ'' lymphoma [15], but rather as an abnormal immune response possibly related to an increased risk of developing a lymphoma [7, 11]. In fact, two of the eight cases described by Nam-Cha et al. [9] developed NHL in relatively short period; moreover, some of the follow-up of the other reported cases are too short to rule out this possibility in the future, thus suggesting the need for further studies and prolonged follow- up to better clarify this aspect.
The presented cases observed in HIV patients, as the others [7, 9, 11] probably represent an ''excessive'' response of normal oligoclonal germinal centres.
Recently, the involvement of B cell expansion also in the development of chronic graft-versus-host disease (cGVHD) has been documented after allogeneic stem cell transplant (HSCT) suggesting the use of B cell-depleting therapy with rituximab for the management of this long-lasting life-threatening complication after HSCT [27-31]. From a cytological point of view, the differential diagnosis between follicular hyperplasia and follicular lymphoma may be difficult or even impossible without ICC, FC, or PCR. In fact, FL may show a relatively polymorphous background, as in our cases, even with numerous follicular centre cells; a proliferative index may not be determinant for the differential diagnosis. As for FC, CD19/10 in routine practice, CD19/10 co-expression with a definite light chain restriction is generally sufficient to diagnose FL.
From a clinical point of view, the first case was further complicated by the fact that the patient had also suffered from FL, which inevitably influenced the final cytological and FC interpretation.
As to the technical approach, FC applied to FNC is routinely used in the diagnosis and follow- up of lymphoproliferative processes and leads to a correct diagnoses in most of the cases. We think that the present cases do not reduce the value of FNC, FC, or other radiological procedures in the diagnosis and management of lymphoadenopathies; instead, they suggest greater attention on data evaluation and clinical

correlation in patients suffering from autoimmune or immunodeficiency syndromes. At the same time, none of the techniques reported earlier has an absolute diagnostic value; hence a histological evaluation should be performed in the follow-up of lymphoid disorders in all the cases carrying discrepant clinical and instrumental data.

Keywords: Lymph node, light chain restriction, flow cytometry, acquired immunodeficiency syndrome.

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SUMMARY

Clonal B-cell populations in non-lymphomatous processes have been sporadically reported in enlarged reactive lymph nodes and mucosa-associated lymphoid cell populations.
These generally small clones are considered non-malignant proliferations of B-lymphocytes determined by an abnormal response to bacterial or viral antigen stimulation.
In cases reported in literature, clonality was detected by light chain assessment and or by polymerase chain reaction (PCR) analysis of immunoglobulin heavy chain (IgH) gene in histologically and clinically proven non lymphomatous processes.
In this study the clinical, cytological, phenotypical and pathological features of three HIV patients in which non-lymphomatous clonal B-cell populations detected in enlarged lymph nodes are reported. All the patients complained for later cervical lymph nodes enlargement, positive at the FDG-positron emission tomography scan.
Fine needle cytology, coupled with flow cytometry showed atypical lymphoid cell proliferations and kappa (2 cases) or lambda (1 case) light chain restriction.
Reactive, non lymphomatous nature of these processes were then proven by histological control in two cases and by clinical follow-up in the last one; corresponding clinical and pathological aspects are discussed.
Clonal B-cell populations in non-lymphomatous processes can sporadically occur in enlarged reactive lymph nodes in immunodeficiency as well as in autoimmune processes. Awareness of the phenomenon and attention should be paid in the evaluation of corresponding pathological features and in the clinical management of corresponding patients.

RIASSUNTO

Popolazioni linfocitarie B-cellulari clonali, non linfomatose, sono state sporadicamente osservate in linfonodi reattivi ed in proliferazioni linfocitarie mucosa-associate.
Questi cloni cellulari, generalmente piccoli, sono considerati proliferazioni non maligne espressione di una abnorme risposta immunitaria ad antigeni batterici o virali.
Nei casi descritti in letteratura la clonalità è stata identificata mediante valutazione delle catene leggere o del gene delle catene pesanti IGH mediante polymerase chain reaction (PCR).
In questo studio sono riportati gli aspetti clinici, citologici e fenotipici di tre pazienti HIV positivi in cui sono state identificate popolazioni linfocitarie B-cellulari clonali, non linfomatose. I tre pazienti avevano evidenziato ingrandimento di linfonodi laterocervicali, positivi alla FDG-positron emission tomography scan e furono sottoposti ad una biopsia per ago sottile. L'esame citologico e citofluorimetrico delle corrispondenti sospensioni cellulari evidenziò la presenza di restrizione per le catene leggere kappa (2 casi) e lambda (1 caso).
La natura reattiva, non-linfomatosa di questi processi fu provata dal successivo controllo istologico in due casi e dal follow-up nel terzo.
Popolazioni linfocitarie B-cellulari clonali non linfomatose, possono svilupparsi in linfonodi reattivi in corso di sindromi da immunodeficienza come in processi autoimmuni.
La conoscenza del fenomeno ed estrema attenzione dovrebbero essere utilizzati nella valutazione diagnostica dei corrispondenti aspetti fenotipici e nel trattamento clinico dei pazienti corrispondenti.

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