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Difficult Decisions

Lymphocytosis: is it leukaemia and when to treat

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The causes of lymphocytosis range from the benign self-limiting response seen in certain infections to frank malignancy (Table I). It is usually possible to differentiate benign from malignant lymphocytosis on the basis of the clinical picture, the blood film, and a small number of widely available investigations. The further investigation of the few remaining cases has been greatly aided by recent developments in immunophenotyping, chromosome analysis and the detection of immunoglobulin and T-cell receptor gene rearrangement. The identification of monoclonality is not an absolute indication for treatment and the decision whether to undertake this in an individual patient must be based on the clinical picture as well as the nature of the lymphocyte involved.

Investigation of lymphocytosis

The investigation of lymphocytosis is largely determined by the morphological appearance of the cells. The lymphoblasts seen in acute lymphoblastic leukaemia (ALL) rarely cause confusion with proliferations of mature cells and this article is primarily concerned with differentiation of the reactive lymphocytoses from the chronic lymphoproliferative disorders.

Clinical picture

The age of the patient, the duration of symptoms and a history of bleeding, recurrent infections or symptoms of anaemia will provide a substantial insight into the likely underlying cause. Chronic, malignant lymphocytoses are rare in children and young adults, when the commonest causes of lymphocytosis are infective. Such infections may be associated with non-specific malaise or more specific symptoms such as the characteristic whoop in *Bordetella pertussis* infections or an ampicillin-induced rash in glandular fever. Important physical findings are jaundice, lymphadenopathy, hepatosplenomegaly, petechiae, purpura or signs of infection. While all of these are commoner in the malignant lymphocytoses each may be seen in infectious mononucleosis, and cervical lymphadenopathy may occur with a variety of viral infections. Skin infiltration may suggest a T-cell proliferation since malignant T-lymphocytoses are frequently dermotrophic. Fundoscopy is important to exclude haemorrhage, signs of hyperviscosity or chorioretinitis in toxoplasmosis. It is however worth noting that malignant lymphocytosis may initially be diagnosed on a routine blood count with no specific symptoms.

Blood count

The normal lymphocyte count varies with age (Table II). A lymphocytosis is conventionally defined as a lymphocyte count above 4.0×10^9/l in adults or 9×10^9/l in infants. Peripheral blood lymphocytes can be divided into several subpopulations by phenotypic and functional analysis (Table II).

The magnitude of the lymphocytosis is often less helpful than the appearance of the cells in determining the underlying cause. Infection is usually associated with counts of $10–40 \times 10^9$/l although they may be as high as 100×10^9/l. In chronic lymphocytic leukaemia (CLL) the count is usually substantially raised ($30–300 \times 10^9$/l), although the diagnosis may be suspected with counts as low as $5–10 \times 10^9$/l if their morphological appearance is characteristic. Lymphocytosis of this magnitude in conjunction with marked lymphadenopathy or hepatosplenomegaly is, however, more likely to be

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Table I Causes of lymphocytosis

Reactive		
Infections: Viral:	Infectious mononucleosis. (Epstein-Barr virus)	
	cytomegalovirus (CMV)	
	infectious lymphocytosis (Coxsackie).	
	occasionally mumps, varicella, hepatitis, rubella, influenza	
Bacterial:	Pertussis	
	occasionally cat-scratch fever, tuberculosis, syphilis, brucellosis.	
Protozoal:	Toxoplasmosis	
	occasionally malaria.	
Auto-immune:	rheumatoid arthritis	
Endocrine:	thyrotoxicosis, Hashimoto's disease	
	hypopituitarism	
Carcinoma		

Malignant	Relative incidence of B- and T-lymphocyte phenotypes B	T
*Chronic lymphocytic leukaemia (CLL)	+++	+
Prolymphocytic leukaemia (PLL)	++	+
Hairy cell leukaemia (HCL)	+++	−
*Non-Hodgkin's lymphomas (NHL) (including adult T-cell leukaemia lymphoma (ATLL))	++	+
†Cutaneous T-cell lymphocytosis (CTCL)	−	+++

*B-CLL and NHL are relatively common. All others are rare; †CTCL = Sezary syndrome and mycosis fungoides.

due to low grade lymphoma with overspill into the blood.

The haemoglobin, platelet and neutrophil counts may be of benefit in differentiating primary and reactive lymphocytoses. Malignant causes, especially in the advanced stages, are more likely to be associated with cytopaenias. Hairy cell leukaemia (HCL) classically presents with cytopaenia rather than a raised lymphocyte count. Conversely, infections such as infectious mononucleosis can be associated with anaemia or thrombocytopaenia due to autoimmune destruction.

Blood film

Small mature lymphocytes may be seen in both malignant lymphocytoses such as CLL and in reactive states such as whooping cough, although the presence of numerous smear cells is highly suggestive of CLL. A relatively heterogeneous population of lymphocytes is more characteristic of the non-Hodgkin's lymphomas (NHLs). The large atypical lymphocytes seen in infectious mononucleosis have evident nucleoli and basophilic cytoplasm and are more likely to be mistaken for blast cells. They are, however, reactive $CD8^+$ T-lymphocytes and are usually fairly easily distinguished by their lobulated nuclei, mature condensed chromatin pattern and foamy cytoplasm. Cells with similar appearance are seen in cytomegalovirus and toxoplasma infections. Rarer cell types with a distinctive morphological appearance include pro-lymphocytes, hairy cells and Sezary cells.

It is difficult to distinguish between lymphocyte subsets on the basis of appearance alone. The malignant T-cell disorders, however, tend to have more convoluted nuclei and may contain azurophilic granules. The latter may also be seen in the non-T, non-B large granular lymphocytes or natural killer (NK) cells.

Table II Normal peripheral blood lymphocytes

	Variation of count with age *Normal range* $\times 10^9/l$
Birth	3.5–8.5
6 years	5.5–8.5
12 years	1.5–4.0
Adults	1.5–4.0

Lymphocyte subsets	*Percentage of lymphocytes*
T-lymphocytes	60–75%
T helper (CD4+)	40–55%
T suppressor (CD8+)	20–25%
B-lymphocytes	5–15%
Non-T- non-B-cells	5–25%

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Further laboratory investigations

A limited number of further investigations will exclude the majority of reactive lymphocytoses (Table III). Abnormalities in hepatic function may reflect infiltration but are also extremely common in infectious mononucleosis and raised transaminases in particular should provide a useful diagnostic pointer. The heterophile agglutinin test (or the Monospot modification) is dependent on the presence of IgM antibodies to the Epstein–Barr virus. The Monospot is negative in cytomegalovirus (CMV) and other infective lymphocytoses and isolation of an organism or the demonstration of a rising antibody titre should aid diagnosis in these cases.

Hypercalcaemia is suggestive of a malignant aetiology and is particularly common in adult T-cell leukaemia lymphoma (ATLL), an aggressive disorder usually seen in patients of Japanese or Caribbean origin and associated with human T-cell leukaemia virus (HTLV) 1 infection.

The presence of an M band on serum protein electrophoresis or immunoparesis is likewise suggestive of a monoclonal disorder.

Further investigation involves determination of the lineage of lymphocyte involved or the demonstration of monoclonality. Immunophenotyping and chromosome analysis are now widely available but analysis of gene rearrangement is still restricted to specialist centres.

Immunophenotype

Different lymphocyte subpopulations can be defined by their expression of cell surface antigens determined by red cell rosetting and a variety of polyclonal and monoclonal antisera (Table IV). (For review see reference 1.) Staining with monoclonal antibodies (McAbs) will usually determine the lineage of the predominant lymphocytes but will not in general define monoclonality. Reactivity can be ascertained using sandwich techniques with fluorescent anti-mouse immunoglobulin or by immunoperoxidase/alkaline phosphatase staining. The immuno-enzymatic methods using blood smears are often more convenient for a routine laboratory[2] but the fixation used may destroy some antigens. Fortunately this is not the case for those antigens of most value in the classification of lymphocytes.

Table III Investigation of lymphocytosis

1. Full blood count and examination of blood film.
2. Liver function tests.
3. Monospot/heterophil agglutinin test.
4. Calcium and bone chemistry.
5. Thyroid function tests and autoantibodies.
6. Serum protein electropheresis and immunoelectropheresis.
7. Chest X-ray.

B-cells may be detected with B-cell specific monoclonal antibodies or by the detection of surface immunoglobulin (SmIg). In practice a chronic lymphocytosis composed of B-cells is almost invariably malignant and B-cells expressing receptors for mouse erythrocytes or the CD5 (T1) antigen are usually due to CLL. B-cells may express more than one type of heavy chain with simultaneously μ and δ chain expression being particularly common. Any one clone of B-cells is however restricted to expression of only one light chain type. The ratio of κ/λ light chain-bearing cells is approximately 2:1 although the normal range is wide. Deviation outside this range is seen in monoclonal B-cell disorders mature enough to express SmIg and has provided one of the best indicators of malignancy. Using these techniques it is also possible to detect minority monoclonal populations in disorders such as the low-grade lymphomas, when the blood count and film appear normal.[3] Studies of SmIg may occasionally be difficult to interpret due to passive absorption of immunoglobulin by Fc-bearing cells and low-level expression of SmIg in some CLLs and the less differentiated NHLs. Analysis of gene rearrangement may then be appropriate.

T-cells can be defined by rosetting with sheep red cells or by monoclonal antibody reactivity. Phenotypic subsets, e.g. CD4+ CD8−(helper/inducer) or CD8+ CD4−(cytotoxic/suppressor) correspond in general to different functional subsets although this is not invariable. The different types of chronic T-lymphoproliferative disorders can be defined by the morphological appearances and McAb staining (Table IV).

Many non-T- non-B-lymphocytes are large granular lymphocytes that have natural killer activity, express Fc receptors for IgG and antigens associated with NK cells such as NHK-1.[4] Lymphocytoses of such cells are exceedingly rare.

Chromosome analysis

Clonal, karyotypic abnormalities are less commonly detected in chronic lymphoproliferative disorders than in the acute leukaemias or chronic granulocytic leukaemia, reflecting the relatively low mitotic index in these cells. The use of T- and B-cell

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mitogens and trypsin-giemsa banded staining techniques have increased the yield of analysable metaphases and it is possible to detect chromosomal abnormalities in several varieties of lymphocytic leukaemia. Abnormal clones have been demonstrated in 20–35% of B-CLL where trisomy 12 and translocations involving the long arm of chromosome 14 (14q) appear most common.[5,6]

B-PLL appears to show a better response to mitogens with abnormalities seen in approximately half the cases analysed.[7] Analysis of involved lymph nodes in low and high grade B-NHL yields karyotypic abnormalities in over 95% of cases and may therefore aid diagnosis in cases where lymphocytosis and lymphadenopathy coexist.[8]

Analysis of T-cell disorders demonstrates clonal abnormalities in approximately half of cutaneous T-cell lymphomas (CTLC) (Sezary syndrome and mycosis fungoides), the majority of T-NHL nodes and in a proportion of T-CLLs.[6,8]

It is worth noting that band q32 on chromosome 14 contains the gene coding for the immunoglobulin heavy chain and translocations involving this locus are the most common non-random abnormalities reported in a variety of low and high grade B-cell tumours. They are also found, although less commonly, in the malignant T-cell lymphocytoses.[6]

Glucose-6-phosphate dehydrogenase (G6PD)

This X-linked enzyme can be used to demonstrate clonality in patients heterozygous for electrophoretically distinct isoenzymes of G6PD. Such variation is, however, rare in Caucasian populations and its applicability is therefore limited in practice to heterozygous negro women with lymphoproliferative disorders. Isoenzyme analysis has been used to demonstrate that the clonal proliferation in CLL is restricted to lymphocytes whereas in chronic granulocytic leukaemia it involves myeloid, erythroid and lymphoid lineages, reflecting a stem cell defect.[9]

Gene rearrangement

It is occasionally not possible to demonstrate monoclonality in B-cell proliferations using the above techniques and in the T-cell proliferations uniformity of phenotype as determined by monoclonal antibodies is not necessarily indicative of a monoclonal population. The demonstration that genes coding for the immunoglobulin heavy and light chains in B-cells and the T-cell receptor for antigen (TCR) in T-lymphocytes undergo rearrangement early in cell differentiation has provided a powerful additional method for the detection of clonality in such cases.[10,11] These genes have several structural and functional properties in common. In their germ line form they exist as discontinuous genetic elements consisting of multiple subsegments coding for constant and variable regions. The latter are subdivided into variable (V), diversity (D) and joining (J) segments. During lymphocyte differentiation rearrangement of the genes for the appropriate receptor is mandatory and is achieved by deletion of segments of DNA. Rearrangement of the immunoglobulin heavy chain

Table IV Immunophenotype of normal and malignant lymphocytes

B-lymphocytes	*SmIg*	*k/λ restriction*	*M. rosettes*	*CD20(B1)*†	*CD5(T1)*	*HLA II(DR)*
Normal	+	−	−	+	−	+
B-CLL*	+/−	+	++	+	+	+
B-PLL	++	+	−	+	+/−	+
HCL	+	+	+/−	+	−	+
B-NHL	+/−	+	+/−	+	−	+

T-lymphocytes	*E. rosettes*	*CD2(T11)*	*CD3(T3)*	*CD4(T4)*	*CD8(T8)*	*CD5*	*CD7(3A1)*	*HLA II*‡
Normal	+	+	+	2/3+	1/3+	+	+	−
T-CLL	+	+	+	−	+	+/−	+/−	+
T-PLL	+	+	+	+	−	+	+	−
CTCL	+	+/−	+	+	−	+	−	+
ATLL	+	+/−	+	+	−	+/−	+/−	+/−

*For abbreviations see Table I. †CD=cluster of differentiation as defined at the three Workshops on Leukocyte Differentiation. ‡Normal activated T-cells express HLA II and testing with anti-HLA II antibodies may therefore be positive in viral lymphocytoses.

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occurs in two steps: a D region is first brought into proximity to the J region and then a V region gene is added to this to give a V–D–J rearranged gene combined to the constant portion of the gene. Messenger RNA is then produced resulting in the synthesis of μ-heavy chains and their appearance in the cytoplasm. These cytoplasmic $\mu+$ cells are known as pre-B-cells. Following rearrangement of the heavy chain the κ or λ light chain rearranges by a similar process with the elaboration of light chain peptides and the formation of complete immunoglobulin. This becomes incorporated as surface-bound immunoglobulin in the membrane of the mature B-cell.[12] An analogous situation exists in rearrangement of the α, β and γ components of the TCR complex, with β and γ rearrangements preceding that of the α chain. As in B-cells these rearrangements are coordinated with alterations in cell phenotype. Transcription of the β gene concurs with surface expression of CD5(T1) and CD2(T11) and Tα chain gene transcripts antedate the appearance of surface CD3(T3).[13]

These rearrangements lead to changes in the location of restriction endonuclease sites and consequently in the size of DNA fragments produced by digestion with these enzymes. Separation of the fragments by electrophoresis, Southern blotting and subsequent hybridization with labelled DNA probes homologous with subsegments of either the immunoglobulin or TCR genes allows detection of clonal populations when these represent as little as 1% of the total DNA.[14] These appear as a band on a Southern blot distinct from that seen with germ line DNA. Polyclonal lymphocytoses yield fragments of widely varying size with each being below the level of sensitivity of detection and therefore not visible on autoradiography.

These techniques can be utilized to define monoclonality, to assign a neoplasm to T- or B-cell lineage and to detect small numbers of malignant cells not evident by morphological assessment. Since the immunoglobulin heavy chain and the constant region of the β chain of the TCR (cβTCR) genes rearrange prior to expression of SmIg or CD3 respectively it is possible to demonstrate monoclonality in relatively immature populations. It is now well recognized for example that the majority of 'non-T, non-B' ALLs and 'null' cell NHLs are clonal B-cell proliferations.[12,15]

They have also been used to demonstrate that relatively 'benign' disorders such as the CD8+ γFc receptor+ T-cell proliferations are clonal in the majority of cases.[16] These rare disorders are associated with cytopaenias and often with rheumatoid arthritis, and their primary or reactive nature has been controversial.[17] Likewise, distinguishing between advanced mycosis fungoides and dermatopathic lymphadenopathy can be difficult. Both are associated with chronic skin disease, lymphadenopathy and the presence of abnormal cells in the peripheral blood. In advanced mycosis fungoides clonal cells can be detected by analysis of cβTCR rearrangement in the blood, skin and lymph nodes, thus facilitating differentiation from the reactive lymphocytes seen in dermatopathic lymphadenopathy.[18]

When to treat

The presence of a malignant lymphocytosis is not, in itself, an indication for therapy. Nor should the decision to start treatment be based on the magnitude of the lymphocytosis alone. In the low grade proliferations such as B- and T-CLL, hairy cell leukaemia and the low-grade NHLs specific treatment is only necessary if there are symptoms, significant cytopaenias, a rapidly rising cell count or rapidly expanding tumour mass or organ failure. Some patients with early B-CLL and low-grade NHL may demonstrate static disease or even spontaneous regression and early treatment of asymptomatic cases has not been shown to prolong survival.[19,20] In T-CLL, long survival of good quality may be possible without treatment even in those patients who are neutropaenic at diagnosis.[17] It is important to detect the relatively rare mature T-lymphoproliferative disorders (T-PLL, ATLL, Sezary syndrome) since treatment with the adenosine deaminase inhibitor deoxycoformicin may be more effective than standard chemotherapy in these cases.[21]

In the relatively aggressive chronic lymphoproliferative disorders patients are usually symptomatic at diagnosis or soon after and appropriate therapy should be instituted. The lymphoproliferative disorders are treated predominantly by cytotoxic drugs and the precise treatment depends on the particular disease entity. This is beyond the scope of this article.

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