Value of CD15 immunostaining in diagnosing Hodgkin's disease: a review of published literature

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SUMMARY The role of antibodies of CD15 as diagnostic markers of Hodgkin's disease was assessed from a review of the literature. A total of 571 cases of Hodgkin's disease and 386 cases of non-Hodgkin's lymphoma were included. The sensitivity of CD15 in detecting cases of Hodgkin's disease was 80% or 91% if cases of lymphocyte predominant Hodgkin's disease were excluded. The specificity of CD15 was only 80·6%, or in other words, 19·4% of cases of non-Hodgkin's lymphoma were CD15 positive. In an ideal test both the sensitivity and specificity would be 100% and if the test performance were no better than chance then they would both be 50%. It is concluded that CD15 immunostaining cannot be regarded as a sensitive or specific marker of Hodgkin's disease. Application of this formal method of analysis to other immunohistological reagents and panels of antibodies is discussed.

The accurate diagnosis of lymphoid neoplasms has increased in importance in recent years, with improvements in treatment and prognosis. The protean histopathological appearances of Hodgkin's disease are well known[1][2] yet several series have shown the difficulty that this diagnosis can pose to experienced pathologists.[3–5] Cells resembling Sternberg-Reed cells can be seen in reactive conditions and in a variety of lymphoid and non-lymphoid neoplasms.[6–8] The application of immunohistological methods to lymphoid lesions has contributed to our understanding of these disorders and has provided an objective means of confirming tumour lineage.[9][10] These methods have helped predominantly in the diagnosis of non-Hodgkin's lymphomas, although the immunological examination of cases of Hodgkin's disease has provided information regarding its complex cellular composition.[11–13] Unfortunately, there has been less progress in the definition of phenotypic features that allow cases of Hodgkin's disease to be distinguished clearly from those of non-Hodgkin's lymphomas. This distinction is important, not just from the point of view of furthering our understanding of these diseases, but from the practical difference in their management.

The monoclonal antibody Leu M1 was raised against the U-937 histiocytic cell line[14] and recognises a trisaccharide antigen lacto-N-fucopentaose III or the so called "X hapten".[15] Other antibodies that recognise this antigen are grouped or clustered in cluster of differentiation 15 (CD15).[15][16] Several studies have described the reactivity of CD15 with Sternberg-Reed cells[17][18] and have raised the possibility that expression of CD15 could be used to discriminate between Hodgkin's disease and non-Hodgkin's lymphomas. More recently reports of non-Hodgkin's lymphomas staining with Leu M1 have cast doubt on its value as a disease marker.[19–22]

Our initial experience with Leu M1 (Becton Dickinson) was favourable and we found it highlighted Reed-Sternberg cells in polymorphic cellular infiltrates, often picking them out when they were sparse. We interpreted staining with this antibody as corroborating a diagnosis of Hodgkin's disease when that diagnosis was suspected on other grounds, but in an unselected series of 57 cases of high grade non-Hodgkin's lymphoma we found six cases (three B cell, three T cell) that stained with Leu M1. It is in those cases where there is a differential diagnostic problem between high grade lymphoma and Hodgkin's disease that the need for an objective means of distinction is required; thus we decided to review the published literature.

In this review the evidence that CD15 antibodies can be used as markers of Hodgkin's disease is assessed by formal analysis of their immunohistological reactivity as described in the published reports. This review aims to illustrate the problems of

translating published results into clinical practice and to highlight the dangers of using a single marker in pathological diagnosis. The analytical methods we used may be of potential value in the evaluation of other antibodies and thus provide the pathologist with an objective means of interpreting published immunohistological results. Although well known in other branches of laboratory medicine, the following analytical concepts are less widely used in histopathology.

Analytical methods

The evaluation of a test comprises two components. First, an assessment of the test's validity—that is, the extent to which a technique measures what it purports to study—and secondly, an assessment of its reproducibility. The reproducibility itself comprises two components: first, the technical reproducibility of the method; and secondly, the reproducibility of the interpretation of the immunostained section by the pathologist.

The methods used for the assessment of validity have been reviewed elsewhere.[23-26] Any new test should be compared with a reference test or "gold standard" and the results of such a comparison placed in a 2 × 2 contingency table (table 1). From such data a series of parameters (operating characteristics) can be defined:

(i) Sensitivity (a/a + c) or the number of cases with a true positive test result divided by the total number of positive cases as defined by the reference method.

(ii) Specificity (d/b + d) or the number of cases with a false positive test result divided by the total number of cases found to be negative with the reference test.

(iii) The predictive value of a positive test (a/a + b) is a measure of the probability that the target disorder is present given a positive result while the predictive value of a negative test (d/ c + d) is a measure of the probability that the target disorder is absent given a negative result.

(iv) Both these parameters depend on the prevalence of the disorder in the population examined (a + c/N).

Table 1 *Analysis of a test*

New test	Reference test		
	Positive	Negative	
Positive	a True positive	b False positive	a + b
Negative	c False negative	d True negative	c + b
	a + c	b + d	N = a + b + c + d

(v) The accuracy of a test (a + d/N) is a measure of the similarity between the new test and the reference test in defining positive or negative cases. A highly discriminant or accurate test will distinguish two populations with very few false positive or negative results—that is, it will be both sensitive and specific.

(vi) The interpretation of a test result depends on the pretest probability of disease—that is, if a pretest probability is low a negative test result has little influence on the diagnosis while a positive test result has a large effect, or given a high pretest probability, a negative test result has a large influence and a positive test a small influence on the diagnosis. Lastly, the evaluation of the validity of a test is influenced by the spectrum of cases chosen for the population studied and bias can easily enter the analysis.[27]

In an ideal test there would be no false negative and false positive results and thus the sensitivity, specificity, accuracy and both positive and negative predictive values would be 100%. If the test performance was not better than chance at discriminating positive and negative cases defined by the reference test, however, then all parameters of test validity would be 50%.[23]

The prevalence of a disorder in the population being examined is of particular importance. At a given prevalence a unit increase in the sensitivity of a test leads to a bigger increase in the predictive value of a negative result than a unit increase in the specificity. Conversely, at a given prevalence a unit increase in the specificity leads to a bigger increase in the predictive value of a positive result than a unit increase in the sensitivity. As the prevalence of a disorder increases in the population studied, then the efficiency (or accuracy) of the test will increase while the efficiency will fall if the reverse is the case.[23] If the sample size (N) is small then there will be a large error in the estimates of the operating characteristics.[28]

The final point to consider is that the operating characteristics of a test may well vary as the test is used.[29] Why should this be so? Many factors are at play—for example, the population used to define the operating characteristics is probably different to that normally seen; there will be a greater range of individuals using the test with varying degrees of skill; and as the test is more widely used there will inevitably be an increasing number of exceptions and unusual cases that will affect the parameters. Thus it is important to be aware of the limitations of defining operating characteristics and to assess continually the usefulness of a given test.

Review of published cases

Nineteen reports[17-22 30-42] describing series of cases stained with Leu M1 (or an equivalent CD15 anti-

Table 2   *Cases of lymphoma stained with CD15 grouped by histological subtype*

Reference	Hodgkin's disease					Non-Hodgkin's lymphoma			
	Total	*Lymphocyte predominant*	*Mixed cellularity*	*Nodular sclerosing*	*Lymphocyte depleted*	*Total*	*B*	*T*	*Other*
Pinkus[18]	69/73	0/4	25/25	41/41	3/3	5/61	1/37	4/20	0/4
Wieczorek[19]						19/38		19/38	
Sheibani[20]	35/45	1/8	4/5	24/26	4/4	14/58	0/17	12/18	2/23
Hyder[21]	42/42	9/9	18/18	15/15		6/23		6/23	
Kadin[22]						5/9		5/9	
Norton[30]	25/32	0/4	12/14	11/12	2/2	2/19	0/5	2/14	0/5
Frierson[32]	35/36	4/4	3/3	28/29					
Hsu[33]	20/22	0/2	3/3	15/15	2/2	0/27	0/10	0/17	
Forni[34]	3/11								
Dorfman[35]	18/23	2/7	5/5	11/11		1/17			
Kornstein[36]	20/23	3/3	7/10	10/10		7/22			
Jack[37]	43/65	2/8	16/20	21/33	4/4	4/12			
Swerdlow[38]	11/13	0/1	2/2	8/9	1/1	1/18	0/13	1/5	
Stein[39]	62/107	13/53	18/20	25/25	6/9				
Strauchen[40]	11/14								
Myskow[41]	52/60	10/14	23/23	14/17	5/6	4/60	1/42	3/10	0/8
Meis[42]	11/15		3/4	7/8		7/22	2/10	4/10	1/2
Total positive	457	44	139	231	27	75	4	56	3
Total cases	571	117	152	261	31	386	134	164	42

body) were scrutinised. Single case reports were not considered and those reports duplicating cases previously reported were, as far as possible, excluded. The manner of case selection, the nature of the staining pattern, and where possible, the proportion of cells staining were recorded.

Seventeen of the series were evaluable and table 2 shows the result. In some series a detailed breakdown of the subtypes of lymphoma was not given. From these data contingency tables showing all cases (table 3) and all cases excluding lymphocyte predominant Hodgkin's disease (table 4) were constructed. Table 5 gives the calculated parameters of validity.

The proportion of Reed-Sternberg cells staining with CD15 varied greatly between cases in all series. In general, the pattern of staining was similar in all cases of Hodgkin's disease and in those cases of non-Hodgkin's lymphoma that stained—that is, membrane staining with Golgi or globular cytoplasmic staining. Some workers, however, suggested that

cases of non-Hodgkin's lymphoma staining with CD15 do so in a granular manner and can thus be distinguished from Hodgkin's disease,[18 30 36 43] although others have not confirmed this.[21] While the effect of fixation was variable, staining of B5 fixed material being consistently stronger than that of formalin fixed material, the results obtained were similar. Crocker and Burnett[16] suggested that the differences in fixation were significant and may thus explain some of the apparent discrepancies between series.

In many series a detailed breakdown of the subtypes of lymphoma was given. From these data contingency tables showing all cases (table 3) and all cases excluding lymphocyte predominant Hodgkin's disease (table 4) were constructed. The calculated operating characteristics are shown in table 5. It is clear from the results of this analysis that CD15 immunostaining used on its own is neither a specific nor sensitive marker of Hodgkin's disease.

Table 3   *Analysis of a CD15 staining (new test) compared with pathologists' diagnosis (reference test) from pooled data derived from table 2*

New test: CD15	Reference test: pathologist		
	Positive	*Negative*	
Positive	a 457	b 75	a + b 532
Negative	c 114	d 311	c + d 425
	a + c 571	b + d 386	a + b + c + d 957

Table 4   *Analysis of CD15 staining compared with reference test with all cases of lymphocyte predominant Hodgkin's disease censored from data derived from table 1*

New test: CD15	Reference test: pathologist		
	Positive	*Negative*	
Positive	a 413	b 75	a + b 488
Negative	c 41	d 311	c + d 352
	a + c 454	b + d 386	a + b + c + d 840

Table 5 *Results of CD15 analysis from pooled data expressed as percentages*

	All cases of Hodgkin's disease	Lymphocyte predominant Hodgkin's disease excluded
Sensitivity (a/a + c)	80·0	90·9
Specificity (d/b + d)	80·6	80·6
Accuracy (a + d/N)	80·2	86·2
Predictive value of a positive result (a/a + b)	85·9	84·6
Predictive value of a negative result (d/c + d)	73·2	88·3

Is this a fair analysis?

The first major problem of this form of analysis is that the reference test or "gold standard" used is the subjective interpretation of the pathologists concerned. These differ in each series. Studies are often retrospective, cases are selected in different ways, and the manner of case selection is rarely stated. Most of the cases detailed in the CD15 review were probably selected as characteristic morphological examples of lymphoma. Relatively few cases were acknowledged as being difficult diagnostic problems. Furthermore, the prevalence of Hodgkin's disease and non-Hodgkin's lymphoma in the individual or combined series is unlikely to reflect that seen in the course of diagnostic histopathology.

Another important and related problem is whether it is valid to attempt to identify a disease on the basis of expression of a single antigen, especially when the underlying nature of the disease remains unknown, as in Hodgkin's disease. There are precedents for the use of a single marker for the definition of pathological subgroups of disease: the common acute lymphoblastic leukaemia antigen (CALLA or CD10) defines a clinically important subgroup of acute lymphoblastic leukaemia[44] and the presence of immunoreactivity with the antibody Ki1 (CD30) may define a subgroup of large cell non-Hodgkin's lymphoma.[45] With both these antibodies exceptions exist; "aberrant" expression by other tumours occurs in CALLA[9 10]; and Ki1 recognises determinants on Reed-Sternberg cells[13] and is by no means lineage specific.[45]

In the light of the known diagnostic difficulty that Hodgkin's disease often poses, might it be preferable to use the expression of an antigen such as CD15 as the main criterion for diagnosis? It could be argued that this would lead to improved inter- and intra-observer reproducibility but there is no objective evidence to support this at present. Given our poor understanding of the biology of CD15 expression and its relation to lymphoma, the use of CD15 to define Hodgkin's disease seems to be premature. Indeed, Jack *et al*[37] stated that due to its insensitivity (high

false negative rate in their series), "Leu M1 positivity should not become a prerequisite for the diagnosis of Hodgkin's disease".

The false positive results with CD15 in non-Hodgkin's lymphomas, particularly peripheral T cell lymphoma, may, in fact, reflect a similarity in their biology. The initial report of MMA (Leu M1) by Hanjan *et al* showed that the antigen recognised was present on activated T cells.[14] It may be that CD15 expression is a feature of lymphocyte activation[45] and thus can occur in Hodgkin's disease as well as B and (more commonly) T cell lymphomas. Conversely, some of the false negative results are in the lymphocyte predominant form of Hodgkin's disease. The lymphocyte predominant form of Hodgkin's disease has a substantially lower incidence of CD15 expression in several[21 32 36 41] but not all[17 18 35 39] series. This is particularly so if the nodular form of the disease is considered. Some workers have used this as further evidence for suggesting that nodular, lymphocyte predominant Hodgkin's disease is a distinct pathological entity. While this may be so, removal of lymphocyte predominant Hodgkin's disease from the analysis does not greatly improve the operating characteristics of the test (table 5).

A further difficulty with CD15 and the diagnosis of Hodgkin's disease is that not only is the disease ill defined, the clinical importance of the antigen recognised by CD15 is also ill defined. Several studies[20 38 46] have shown that CD15 immunoreactivity is found in a wide range of normal tissues and non-lymphoid neoplasms. This may reflect cross reactivity with related epitopes or expression of the same epitope in a variety of tissues. Whatever the explanation, it illustrates that several aspects of antibody reactivity need to be considered—namely, hapten/molecular specificity; cell/tissue specificity; and disease specificity. Although these are related they are not necessarily identical.

Although the analytical methods that we have used have drawbacks, we feel that the general conclusions are valid. Furthermore, this form of analysis may help put the literature into a perspective from which more rational decisions regarding the utility of immunohistochemical reagents in diagnostic pathology may be made.

Reproducibility of CD15 immunostaining

Another aspect of evaluation of a test relates to its technical reproducibility. Little information is available regarding the interdepartmental reproducibility of immunohistology, although one small study of anti-immunoglobulin staining of the same case by different departments indicated good concordance.[47] We found that a wide range of different methods had

been used and this may have accounted for some differences. A wide range of monoclonal antibodies are now available, often from more than one source (for example, several CD15 reagents are available; Leu M1 Becton Dickinson; Dako M1 Dakopatts; Tu9 Clonab/Biotest Diagnostics; numerous "private" antibodies), and little data are available on their comparability for use in diagnostic pathology.[35] A system of comparison similar to that suggested for virological reagents might be of value.[48]

The difficulties of interpreting the patterns of immunostaining seen with CD15 and other antibodies have been reported previously. It may be that there are differences in staining pattern in Hodgkin's disease and non-Hodgkin's lymphoma, but the published reports offer no definitive explanations on this point. Suffice to say that this is an aspect of immunohistochemistry that requires great care.

The value of panels of reagents

In general, panels of antibodies are considered to be preferable to the use of a single reagent. The use of CD15 antibody together with other reagents may increase its diagnostic accuracy.[43] It may be that in the correct morphological setting the expression of CD15 and the absence of detectable leucocyte common antigen (CD45) is a characteristic phenotype of Hodgkin's disease[35 42 43] but not all have found this to be so.[33 41] Unfortunately, there are not yet sufficient published data to assess critically this possibility.

The application of panels of antibodies has been extensively investigated in tumour diagnosis,[49–51] in neuropathology,[52] and in the analysis of lymphoid and haematological neoplasia.[9–11 53] The combination of tests, as in the use of panels of antibodies, can be analysed considering their operating characteristics[23–26] by "discriminant analysis". This is not without potential problems. Tests may be arranged in parallel (either or both must be positive) or in series (both must be positive) to define some disease as present. In the former combination of tests the sensitivity is increased but with reduced specificity; in the latter the specificity is increased with reduced sensitivity. To summarise Griner[24]; if all tests in a panel are positive or all are negative the panel is helpful, excluding or confirming disease. If one is positive and others are negative (say) then this may lead to confusion. When dealing with immunohistochemistry, the negative result is often a problem, particularly if diagnoses are to be made by the absence of a marker(s). To quote Sloane and Ormerod: "negative results are of course unhelpful".[54]

Is the method of analysis generally applicable?

Leader *et al* recently incorporated assessment of sensitivity and specificity in publications on immunohistochemistry.[55 56] A discussion of the value of carcinoembryonic antigen (CEA) immunostaining and periodic acid Schiff histochemistry (PAS-D) after diastase treatment in the differential diagnosis of mesothelioma from adenocarcinoma in similar terms has recently been reported.[57] Another study showed that while immunostaining with CEA was of some value in the distinction of adenocarcinoma (CEA positive) from mesothelioma (CEA negative), both false positive and false negative results occurred.[58] It should be noted that the numbers in some of these series are relatively small and that small sample size leads to poor estimates of probability in this form of analysis.[28] The formal analysis might usefully be applied to other antibodies such as prostate specific antigen—prostatic acid phosphatase, for example. Similar methods may be applied in other areas of histopathology: Minkowitz *et al* applied them to the assessment of the diagnostic value of "Tru-Cut" needle biopsy of the breast.[59]

Conclusions

Immunohistology is a useful adjunct to conventional methods of histological diagnosis but it cannot be interpreted in isolation. The value of the technique varies considerably with the diagnostic problem and the reagents used. Warnke and Rouse reported an assessment of the limitations and pitfalls in the application of immunohistology in their haematopathology practice.[60] They emphasised that the published reports on CD15 staining in Hodgkin's disease was largely based "on typical cases . . . .and the findings may not be relevant to staining in difficult problem cases". In their reported experience they found that CD15 immunostaining was helpful in only 17 of 36 cases of Hodgkin's disease seen in one year. As our need for objective help from immunohistochemistry is most apparent in "the difficult case"—for example, in the distinction of T cell lymphoma from Hodgkin's disease—and because T cell tumours not uncommonly express CD15, it is clear that CD15 antibodies are of little value in this diagnostic dilemma. Although CD15 immunostaining may provide further information relating to the phenotypic feature of lymphoma in general and Hodgkin's disease in particular, on the basis of the published reports, it cannot on its own be regarded as a diagnostic discriminant of Hodgkin's disease.

Analysis of published data in the way outlined in this review may supplement casual reading of the literature and personal experience. Data derived from

this method might possibly be applied to increase the objectivity of histopathological and immuno-histochemical decision making. Indeed, data produced in this manner could be of value in developing the use of Bayes' theorem and formal decision analysis in histopathology.[61]

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