

Papers

Periodic acid Schiff reaction in childhood lymphoblastic leukaemia

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Abstract

Aims—To assess the prevalence and degree of periodic acid Schiff (PAS) positivity in blast cells from children with lymphoblastic leukaemia (ALL); its association with other disease characteristics; and its clinical importance in predicting the outcome of treatment.

Methods—Marrow slides from entrants to a large United Kingdom multicentre ALL trial (UKALL X) were batch processed and assessed blind for PAS positivity by one morphologist. Patients were classified into groups A, B, and C, corresponding to less than 1% PAS positive cells, 1–10%, and over 10%, respectively. Their PAS pattern was then compared with other clinical and pathological features of ALL and with treatment outcome.

Results—Slides from 921 children were examined of which 371 (40%) were categorised as group A, 324 (35%) as group B, and 226 (25%) as group C. There was a clear association between the presence of blast cell vacuoles on Romanowsky staining and PAS positivity. Group A (PAS negative) patients included a disproportionate excess of those with L2 morphology, those under 2 or over 6 years of age, those with an initial white cell count over $50 \times 10^9/l$, those with a T or null cell immunophenotype, and those with chromosomal abnormalities other than "high hyperdiploidy". Four years from diagnosis, group C patients had an 8% disease free survival advantage over those in group A ($2p = 0.01$). This was irrespective of initial white cell count, but not of immunophenotype or the presence of vacuoles.

Conclusions—Strong PAS positivity is a feature of "common" ALL and is particularly associated with blast cell vacuoles. It does occasionally occur in other disease subtypes with or without vacuoles. It predicts a better response to current treatment, but not independently of other cell characteristics.

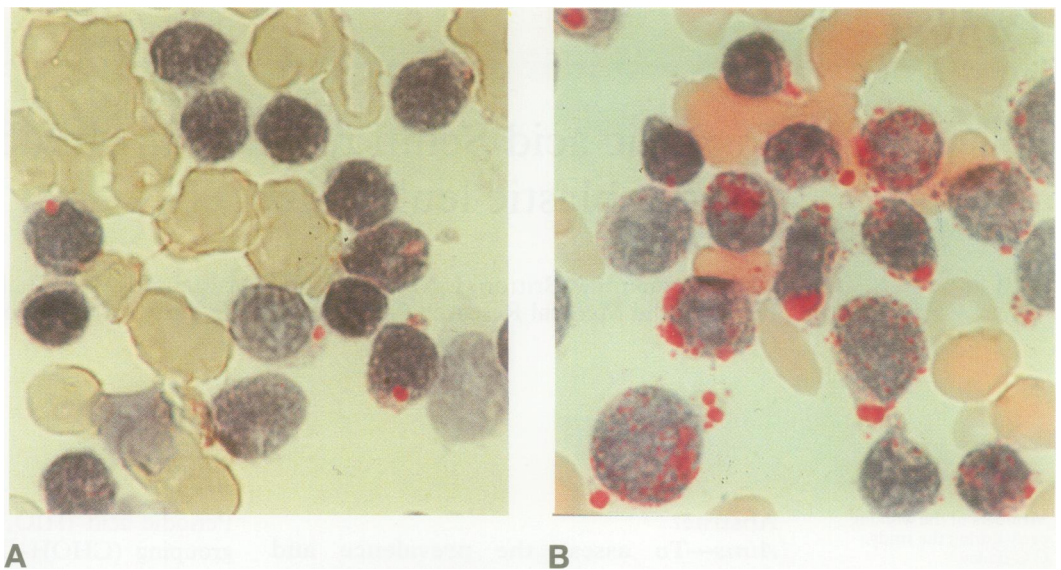
Periodic acid (HIO₄) oxidises the 1–2 glycol grouping (CHOH-CHOH) or its amino- or alkylamino-derivatives to produce a di-aldehyde that gives a substituted magenta dye with Schiff's leucobasic fuchsin. This periodic acid-Schiff (PAS) reaction can be used on a variety of microscopically examined tissues. It detects several classes of naturally occurring carbohydrates, but in the context of blood cells it usually indicates the presence of glyco-

gen. For many years the reaction has been used in the diagnosis and classification of haematological malignancies, and until recently in helping to distinguish between poorly differentiated acute leukaemias where the basic lineage (lymphoid or myeloid) was morphologically unclear. Classic "block" PAS positivity has always been a reassuring (though unspecific) hallmark of lymphoblasts. Lately, though, the advent of immunophenotyping and genetic analysis has largely made the diagnostic value of PAS staining redundant except where these newer techniques are unreliable or unavailable.

Apart from its use in diagnosis, however, because childhood lymphoblastic leukaemias (ALLs) show a highly variable pattern and intensity of staining, there have been several attempts to assess whether PAS pattern relates in any way to response to treatment or disease subtype. Most early studies exploring its prognostic value found that children who had positive blast cells fared better.¹⁻⁶ Other contemporary reports disagreed,⁷⁻¹⁰ but more recently a large group of children from the United Kingdom Medical Research Council (MRC) trial UKALL VIII showed that there was a small but significant survival advantage for the 55% who had more than 1% PAS positive blast cells. Not only that, but the effect on survival seemed to be at least partly independent of other major prognostic factors, including age, sex, and diagnostic white cell count.¹¹

As far as the association between PAS pattern and identifiable subtypes of ALL is concerned, fewer data are available. Five years ago a consecutive cohort of 87 children indicated strong positivity to be associated with the phenotype of "common" or pre-B ALL,

Figure 1 Variable pattern of PAS reaction in childhood ALL: (A) Occasional "block" PAS positivity in 1–10% of cells. (B) Strong multiple blocks of PAS positive material in most blast cells.



but rare in other immunologically defined groups.¹¹

The present study, the largest to date, was undertaken for two reasons. First, to explore further the association between PAS pattern and currently recognised clinical, morphological, immunological, and cytogenetic features of childhood ALL; and secondly, to see if positivity still retains any prognostic importance with more modern treatment programmes.

Methods

The children studied were those participating in UKALL X, a United Kingdom national Medical Research Council (MRC) trial for all children with any type of ALL (other than B cell ALL) that was open for entry from 1985 to 1990. The trial design and therapeutic protocol have been described elsewhere.¹² One of the trial requirements was the submission of diagnostic bone marrow slides for central review of morphology, according to the French American British (FAB) criteria for the classification of ALL.¹³ Where spare Romanowsky stained slides were available, they were batched and subjected to the PAS reaction using the technique of Hayhoe *et al*¹⁴

and examined fresh by one observer (JSL) who scored the result into one of three groups: A, where less than 1% of blasts showed any positivity; B, where 1–10% cells were positive; and C, where over 10% of cells were strongly positive. The appearances of categories B and C are illustrated in fig 1. Within group A complete PAS negativity was only scored in slides where occasional positive neutrophils functioned as internal positive controls. All slides were carefully refiled for future use for other purposes.

Children were excluded only if no slide was available for study. PAS scoring was done without knowing any of the other features of each patient's disease except the morphological features of the blast cells. Similarly, the scoring was prospective—without knowledge of any patient's response to treatment.

FAB morphology was assessed by a panel of three haematologists, as described before,¹⁵ and also included the assessment of the presence or absence of vacuoles in the blast cell cytoplasm. Immunophenotype was based on data supplied by individual centres participating in the MRC trial. "Common" ALL was defined as CD10+ and CIG-, pre-B as CD10+ and CIG+, T ALL as showing unequivocal T cell lineage markers, and null-ALL as non-T, Tdt+, CD10-, and CIG-. Data on blast cell ploidy were supplied by individual centres. "High hyperdiploidy" was defined as over 50 chromosomes.

The association between PAS reaction and other clinical and pathological features of ALL was tested using simple two-tailed χ^2 statistics. Survival comparisons were made using conventional life table methods and the log rank test.

Results

Of a total of 1613 children entered into UKALL X, 921 (57%) had spare marrow slides available for central review of blast cell PAS staining. There were no data to suggest

Table 1 PAS reaction *v* age, gender, and diagnostic white cell count (WBC)

	PAS positive			Total
	<1%: group A	1–10%: group B	>10%: group C	
All patients	371 (40)	324 (35)	226 (25)	921
Males	221 (42)	177 (34)	123 (24)	521
Females	150 (38)	147 (37)	103 (26)	400
Age 2–6 years	202 (38)	186 (34)	153 (28)	541
Other ages*	169 (45)	138 (37)	73 (19)	380
WBC <10	161 (38)	148 (35)	116 (27)	425
WBC 10–50	112 (37)	122 (39)	72 (24)	306
WBC >50*	98 (52)	54 (28)	38 (20)	190

Figures in parentheses represent the percentage of patients in the individually clinically defined groups.

*Significantly different pattern of PAS positivity from the group as a whole (see text).

Table 2 PAS reaction v cell morphology (FAB type and presence/absence of vacuoles)

	PAS positive			Total
	<1%: group A	1-10%: group B	>10%: group C	
Type L ₁	304 (38)	292 (37)	203 (25)	799
Type L ₂ *	58 (57)	23 (23)	20 (20)	101
Vacuoles >10%*	49 (18)	111 (40)	117 (42)	277
Vacuoles <10%*	313 (50)	204 (33)	106 (17)	623
Total	362 (40)	315 (35)	223 (25)	900

Numbers in parentheses represent the percentage of total patients with a given FAB type or vacuole status; 21 patients were excluded (slides of too poor quality for full morphological assessment).

*Significantly different pattern of PAS positivity from the group as a whole.

Table 3 PAS reaction v immunophenotype

	PAS positive			Total
	<1%: group A	1-10%: group B	>10%: group C	
Common	253 (36)	254 (36)	198 (28)	705
Pre-B	22 (38)	22 (38)	13 (24)	57
T*	58 (70)	20 (24)	5 (6)	83
Null*	16 (47)	16 (47)	2 (6)	34
Total	349 (40)	312 (35)	218 (25)	879

Numbers in parentheses represent the percentage of total patients with that immunophenotype; 42 patients were excluded (no immunological data).

*Significantly different pattern of PAS positivity from the remainder.

Table 4 PAS reaction v blast cell ploidy status

	PAS positive			Total
	<1%: group A	1-10%: group B	>10%: group C	
Normal karyotype	76 (43)	65 (37)	36 (20)	177
Hypodiploidy	7 (50)	3 (21)	4 (29)	14
High hyperdiploidy*	40 (31)	54 (42)	35 (27)	129
Other abnormalities	74 (50)	39 (26)	35 (24)	148
Total	197 (42)	161 (34)	110 (24)	468

Numbers in parentheses represent the percentage of total patients with that ploidy status; 453 patients excluded (no cytogenetic data).

*Significantly different pattern of PAS positivity from others with abnormal karyotypes.

that the remainder differed in terms of their type of ALL or response to treatment.

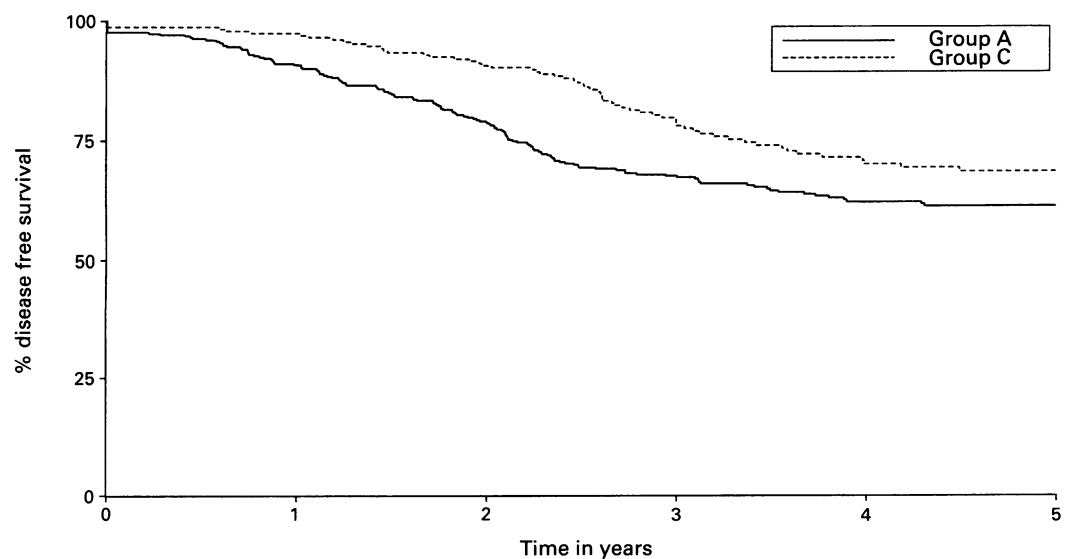
Most patients (550, or 60%) had more than 1% PAS positivity, with 226 (25%) showing a strong reaction in over 10% of cells. There seemed to be an association between the number of cells showing PAS positivity and the number of positive blocks or granules that such cells contained (fig 1), but that observation was not formally tested. The pattern was independent of gender, but not of age or diagnostic white cell count, as can be seen from table 1. Comparing the patients in PAS group C with those in group A, children under 2 or over 6 years old were more likely to be negative ($\chi^2 = 10.23$; $p < 0.005$), as were those with diagnostic white cell counts in excess of $50 \times 10^9/l$ ($\chi^2 = 7.36$; $p < 0.01$).

When studied against blast cell morphology, PAS group C reactions were less common in the 11% of children with L2 disease ($\chi^2 = 5.94$; $p < 0.03$), but much more common in the 31% with cytoplasmic vacuoles in over 10% of cells ($\chi^2 = 102.9$; $p < 0.0001$). The figures are displayed in table 2.

PAS pattern v cell immunophenotype is shown in table 3. A negative reaction was more common in children with T ALL or null ALL when compared with the others ($\chi^2 = 29.6$; $p < 0.0005$). The ploidy state of blast cells and differing patterns of PAS reaction are shown in table 4. Those showing "high hyperdiploidy" seemed to include proportionately fewer PAS negative patients ($\chi^2 = 3.93$; $p < 0.05$).

The disease free survival of 371 PAS group A (negative) children was significantly worse than that of 226 PAS group C (strongly positive) (8% four years from diagnosis; two $p = 0.01$ (log rank)) (fig 2). This difference remained significant if the analysis was stratified by diagnostic white cell count (two $p = 0.03$), but was not independent of cell morphology as a prognostic indicator (specifically the presence or absence of vacuoles in the blast cell cytoplasm) or of cell immunophenotype.

Figure 2 Disease free survival for 226 children in group C (strong PAS positivity in more than 10% of blast cells) compared with that of 371 in group A (less than 1% PAS positive cells).



Discussion

The PAS pattern seen in the UKALL X children confirms and extends the impressions gained from those in UKALL VIII and the Sheffield cohort studied five years ago.¹¹ Strong positivity is seen more frequently in typical "common" ALL arising in 2 to 6 year olds, with a low presenting white cell count and more than 50 chromosomes in the blast cells. It is also strongly associated with the presence of cytoplasmic vacuoles, the single most important morphological feature in terms of predicting response to treatment.¹⁵ Conversely, PAS negative blasts are seen more often in T ALL and null ALL.

None of these categories is mutually exclusive, however, and there are (for example) 17% of children without cytoplasmic vacuoles and 6% of those with T or null ALL who have a type C (strongly positive) PAS reaction. But to say "strongly positive" is perhaps to oversimplify the matter, as the staining pattern can vary from coarse blocks of PAS positive material to fine stippling, and these more subtle distinctions may possibly discriminate between cell types more exactly.

What, precisely, PAS positive material in lymphoblasts is, or what it signifies, is not clear. The chief substance being detected is probably glycogen as the reaction is normally inhibited by salivary amylase. But why, in some children, blasts should be stuffed with glycogen and in others not; why the phenomenon is seen more frequently in some types of ALL and not in others; and why the presence of glycogen-rich cells should be associated with a better response to treatment has yet to be explained. Numbers of PAS type C patients with T or null cell phenotypes are too few to answer the question whether abundant cytoplasmic glycogen is a favourable feature independent of immunological disease type. Similarly, there are as yet insufficient data to explore fully the association between the PAS reaction and cytogenetic abnormalities beyond a broad look at ploidy status.

So what use does the PAS reaction have in the current investigation and classification of childhood ALL? Firstly and obviously it can still be helpful in the recognition and distinction of ALL where immunophenotyping is impossible or the results are confusing. Quality control problems still cause real difficulties in immunological cell typing, and the tests are not available in all laboratories involved in the diagnosis of leukaemia. "Block" PAS positivity in the context of acute leukaemia very seldom occurs other than in ALL, and a type C PAS reaction in a 3 year old child with L1 ALL and a low white cell count almost invariably indicates the "common ALL" immunophenotype.

Secondly, and more tentatively, within a defined prognostic subgroup of ALL (such as children with a diagnostic white cell count of

more than $50 \times 10^9/l$), the PAS reaction may, even in the absence of other outcome-related features, indicate a modest increased or decreased probability of successful treatment. That predictive value is unlikely, on its own, to influence the choice of treatment in the immediate future, but it might be premature to assume that it never could. Greater understanding of biological and metabolic differences in the blast cells of ALL subtypes might lead in future to a more individual approach to initial treatment rather than the current broad stratifications based on crude clinical variables such as the degree of organomegaly and initial white cell count. If PAS positivity does become important it might be worth exploring a way of quantitating intracellular glycogen in blast cells more precisely, though the simple stain seems sufficient for the present.

So although it is currently of only limited use in the diagnosis of ALL, and of no value at all in treatment planning, the PAS reaction may yet enjoy a renaissance. It is cheap and easy to use. The wholesale discontinuation of its routine application in newly diagnosed patients would be premature.

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