4 Health Protection Agency. Identification of *Streptococcus* species, *Micrococcus* species and morphologically similar organisms. SOP ID 4, Issue 1, 2003. www.phls.org.uk/dir/hg/sops2.htm (accessed 21 October 2004).
5 Reed RP, Sinickas VG, Lewis C, Byron KA. A comparison of polymerase chain reaction and phenotyping for rapid speciation of enterococci and detection of vancomycin resistance. *Pathology* 1999; **31**: 127–32.
6 Winston LG, Pang S, Haller BL, Wong M, Chambers HF, Perdreau-Remington F. API 20 Strep identification system may incorrectly speciate enterococci with low-level resistance to vancomycin. *Diagn Microbiol Infect Dis* 2004; **48**: 287–8.
7 Turenne CY, Hoban DJ, Karlowsky JA, Zhanel GG, Kabani AM. Screening of stool samples for identification of vancomycin-resistant *Enterococcus* isolates should include the methyl-a-D-glucopyranoside test to differetiate non-motile *Enterococcus gallinarum* from *E. faecium*. *J Clin Microbiol* 1998; **36**: 2333–5.
8 Collins MD, Jones D, Farrow JAE, Kilpper-Balz R, Schleifer KH. *Enterococcus avium* nom. rev., comb. nov.; *E. casseliflavus* nom. rev. comb. nov.; *E. durans* nom. rev., comb. nov.; *E. gallinarum* comb. nov.; and *E. malodoratus* sp.nov. *Int J Syst Bacteriol* 1984; **34**: 220–3.
9 Devriese LA, Pot B, Kersters K, Lauwers S, Haesebrouck F. Acidification of methyl-a-D-glucopyranoside: a useful test to differentiate *Enterococcus casseliflavus* and *E. gallinarum* from *Enterococcus faecium* species group and from *Enterococcus faecalis*. *J Clin Microbiol* 1996; **34**: 2607–8.
10 Carvalho MG, Teixeira LM, Facklam RR. Use of tests for acidification of methyl-a-D-glucopyranoside and susceptability to efrotomycin for differentiation of strains of *Enterococcus* and some related genera. *J Clin Microbiol* 1998; **36**: 1584–7.
11 Hanson KL, Cartwright CP. Comparison of simple and rapid methods for identifying enterococci intrinsically resistant to vancomycin. *J Clin Microbiol* 1999; **37**: 815–7.
12 Ramotar K, Woods W, Larocque L, Toye B. Comparison of phenotypinc methods to identify enterococci intrinsically resistant to vancomycin (VanC VRE). *Diagn Microbiol Infect Dis* 2000; **36**:119–24.
13 Devriese LA, Pot B, Collins MD. Phenotypic identification of the genus *Enterococcus* and differentiation of phylogenetically distinct enterococcal species and species groups. *J Appl Bacteriol* 1993; **75**: 399–408.
14 National Glycopeptide-Resistant Enterococcal Bacteraemia Surveillance Working Group. Report to the Department of Health. August 2004. www.hpa.org.uk/infections/publications/pdf/GRE%20bact%surveillance%20final%20Aug04.pdf (accessed October 2004).

Peroxidase activity and nuclear density analysis (PANDA) in the diagnosis of haematological malignancy

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Diagnosis of haematological malignancy relies on the assessment of cellular morphology and immunophenotype (having largely replaced cytochemistry), cytogenetic, molecular, and clinical features. Usually, however, an abnormal full blood count (FBC) is the first laboratory indication that haematological malignancy may be present.

Modern haematology analysers measure an increasing number of parameters in addition to traditional indices in a variety of different ways, depending on the manufacturer. Awareness of these parameters on front-line laboratory instruments, and their ability to offer additional diagnostic clues, is a growing area of interest. The Advia 120 haematology analyser (Bayer Diagnostics, Newbury, UK) uses a combination of cytochemistry and light-scatter measurements to derive its peroxidase activity (PA) and nuclear density (ND) analysis (PANDA) cytograms.

Peroxidase activity is measured using the peroxidase channel. In a heated reaction chamber, red blood cells are lysed with a surfactant, and the white blood cells are fixed using formaldehyde. In the presence of hydrogen peroxide and the chromogen 4-chloro-1-naphthol, cells containing myeloperoxidase form a dark precipitate and are characterised by their light-scatter and light-absorption properties.

Nuclear density is derived from the basophil/nuclear lobularity channel. In a heated reaction chamber, phthalic acid strips the cytoplasm from white blood cells (except basophils). Two-angle light scatter is then used to determine cell size and nuclear density. Together, PA and ND are used to derive the white blood cell (WBC) count and the WBC differential.

In a recent study by d'Onofrio,[1] PA and ND cytograms were used to assess the utility of these parameters to assist in the diagnosis and classification of haematological malignancy, leading to the construction of a PANDA pre-classification grid comprising seven PA and two ND categories (Fig. 1). One hundred and eighty cases were studied, including examples of acute leukaemia, chronic lymphoproliferative and myeloproliferative disorders, as well as cases of infectious mononucleosis and peroxidase-deficient neutrophils (both of which also have abnormal cytograms). With some variation in respective categories, overall accuracy of classification using the PANDA grid was reported to be 91.1%.

Use of pattern-recognition software on the next generation of laboratory computer systems seems increasingly likely. Rather than using relatively simple analyser flagging, future computer systems may integrate and assess parameters such as the PANDA profiles, and even alert the operator to possible diagnoses for further investigation.

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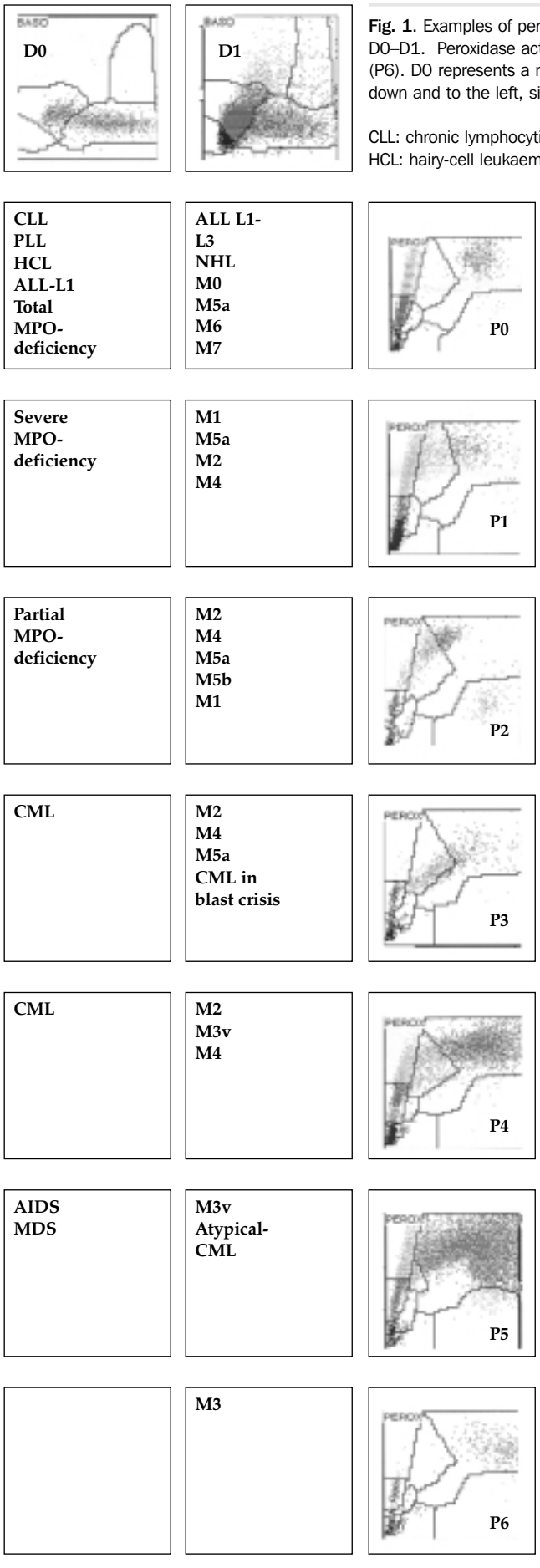

Fig. 1. Examples of peroxidase activity (PA) cytograms P0–P6 and nuclear density (ND) cytograms D0–D1. Peroxidase activity in the predominant cell population varies from absent (P0) to intense (P6). D0 represents a normal ND profile whilst the mononuclear cell population in D1 is shifted down and to the left, signifying the presence of blasts.

CLL: chronic lymphocytic leukaemia, PLL: prolymphocytic leukaemia, HCL: hairy-cell leukaemia, ALL: acute lymphoblastic leukaemia, MPO: myeloperoxidase, CML: chronic myeloid leukaemia, AIDS: acquired immunodeficiency syndrome, MDS: myelodyplastic syndrome, NHL: non-Hodgkin's lymphoma.

Bayer (Tarrytown, NY, USA) has developed an artificial neural network capable of assessing multiple Advia 120 parameters. The system was 'trained' using FBC data from 1000 patients. Initial results are reported to be promising and there are plans to extend the system to include the classification of other disorders such as anaemia.[2]

In the present study, the performance of the PANDA pre-classification grid was assessed retrospectively on 140 randomly selected Advia 120 FBC printouts with abnormal PA and ND cytograms (Table 1) using d'Onofrio's criteria.[1] With the exception of peroxidase-deficient neutrophil (PDN) and infectious mononucleosis (IM) cases, which were validated by biomedical scientists, all diagnoses were made by consultant haematologists.

Of the 18 acute myeloid leukaemia (AML) cases studied, 17 (94.4%) were correctly classified as AML, which compared well with d'Onofrio's results (95.2%). Of the AML cases with a French-American-British (FAB) subtype, all except the two M1 cases were classified correctly. Both M1 subtypes were categorised as probably being either M2 or M4 (P3/D1) using the grid (Table 2).

Importantly, although only one case of M3 AML was present in this study, the characteristic PANDA profile (P6/D1) was displayed. None of the remaining 17 cases of AML exhibited this profile. Indeed, the only haematological malignancy allocated to the P6/D1 category is M3 AML. In view of the response of this disorder to all-*trans* retinoic acid (ATRA), the potential importance of this as an initial screen is highlighted, and has also been noted by others.[3] Unfortunately, d'Onofrio did not subgroup his AML cases according to FAB type, although his pre-classification grid does.

There is some (often considerable) duplication of the FAB subtypes of AML across the pre-classification grid, with, for example, M2 being allocated to P1, P2, P3 and P4 (+D1). Similarly, M5a is allocated to P0, P1 and P2 (+D1). This is not unexpected and reflects the heterogeneity of the disease. Realistically, with the possible exception of M3 AML, the PANDA classification system has the most value as an indicator of a possible case of AML, rather than attempting to pinpoint the FAB classification.

d'Onofrio studied cases of high-grade non-Hodgkin's lymphoma (HG-NHL). The eight NHL cases included in this study were all low-grade (LG), and six had a chronic lymphoproliferative disease (LPD) profile (P0/D0). Clearly, more cases are required to determine whether or not LG-NHL should be allocated to the P0/D0 category of the pre-classification grid.

Table 1. Summary of patients and Advia 120 PANDA results (n=140).

	Number of patients	Number classified correctly	Accuracy (%)
AML	18	17	94.4
ALL	4	4	100
CLL	53	53	100
NHL†	8	0	0
MDS	5	2	40
CMML*	11	2	18
MF*	4	1	25
MPD*	1	0	0
CML	1	1	100
IM†	15	12	80
PDN	17	17	100
Myeloma†	3	0	0
Total	140	109	77.8

*Using P5/D0, †see text for details.
AML: acute myeloid leukaemia, ALL: acute lymphoblastic leukaemia, CLL: chronic lymphocytic leukaemia, NHL: non-Hodgkin's lymphoma, MDS: myelodysplastic syndrome, CMML: chronic myelomonocytic leukaemia, MF: myelofibrosis, MPD: myeloproliferative disease, CML: chronic myeloid leukaemia, IM: infectious mononucleosis, PDN: peroxidase-deficient neutrophils.

The two remaining cases both had a P0/D1 profile. One of these was a case of mantle-cell lymphoma (MCL). Although classified as low-grade, MCL has a broad clinical spectrum, from indolent to aggressive. Examples of the latter often require treatment as for HG-NHL. This case progressed rapidly, despite treatment, and resulted in the death of the patient.

Much variability was noted with the myelodysplasia (MDS), chronic myelomonocytic leukaemia (CMML), myeloproliferative disease (MPD) and myelofibrosis (MF) cases, using P5/D0 for classification. If the pre-classification categories P3 and P4 (+D0) were extended to include the probability of MDS/CMML/MPD/MF in addition to CML, then 64.7% of the cases in this study would have been grouped into these categories. Notably, four of the CMML/MPD/MF cases showed an excess of blasts and the ND cytograms of each had a D1, rather than D0, profile. An excess of blasts can indicate transformation to acute leukaemia and may signify the need for more frequent patient monitoring.

Using the P0/D2 category initially proposed by d'Onofrio, 12 (80%) of the 15 IM cases were classified correctly (compared with 88.2% by d'Onofrio). The D2 profile is not included in the PANDA templates currently issued by Bayer and so these 12 cases would have been excluded from having a high probability of haematological malignancy, as they did not match any of the other pre-classification grid categories. Each was morphologically typical of IM and positive with the Paul Bunnell test. On further investigation, the three remaining cases (P0/D0) were each morphologically typical of IM and positive with the Paul-Bunnell test.

Table 2. Listing of FAB classification of AML patients (n=18).

	Number of patients	Number classified correctly
M0	1	1
M1	2	0*
M2	1	1
M3	1	1
M4	1	1
M5 (a+b)	3	3
M6	1	1
Unclassified	8	7†

*Both classified as AML but not M1.
†one classified as MDS.

Three of the FBC printouts used in the study were from myeloma patients in the leukaemic phase (circulating neoplastic plasma cells in the peripheral blood) and each had a P0/D1 profile. A limitation of the pre-classification grid in its current form is that it does not include this disease entity as a possible diagnosis. More cases are required to assess whether or not leukaemic-phase myeloma should also be assigned to the P0/D1 profile.

In the present study, which included all patient categories, the pre-classification grid was 77.8% accurate, compared to 91.1% in the study by d'Onofrio. By removing cases that do not fall within the current scope of the grid (LG-NHL, MPD, MF, CMML and leukaemic-phase myeloma), overall accuracy increased to 93.8%. Clearly, the pre-classification grid is not exhaustive in its current coverage of haematological malignancy, although more patient data may allow future revision.

In summary, this study supports the findings of d'Onofrio. Furthermore, for cases of acute and chronic leukaemia, the PANDA classification system does appear to have value as a preliminary guide that may allow a more focused approach towards subsequent diagnosis.

The author thanks Teresa Bromidge and Denise Howe for reading the manuscript and suggesting improvements.

References

1 d'Onofrio G. PANDA – Innovative classification of hematopoietic malignancies. *Bloodline Reviews* 2001; **2-R**: 3–6.
2 Zini G, d'Onofrio G. Neural network in hematopoietic malignancies. *Clin Chim Acta* 2003; **333**: 195–201.
3 Lock RJ, Virgo PF, Kitchen C, Evely RS. Rapid diagnosis and characterization of acute promyelocytic leukaemia in routine laboratory practice. *Clin Lab Haem* 2004; **26**: 101–6.