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Performance of an Automated Blood Cell Analyzer with Special Reference to Suspect Flags

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Abstract Performance of the flag system of an automated blood cell analyzer, Coulter STKS, was evaluated. The suspect flags were insufficient for the correct detection of specific abnormalities. The presence of blasts was correctly signaled in most samples (84/86) only if they comprised 20% or more of white blood cells, for example. However, the segregation of blood samples with any important abnormalities (e.g. blasts ≧1%, nucleated red cells ≧1/200WBC, or neutrophils younger than band ≧5%) was possible by adoption of all suspect and definitive flags, because abnormal findings in complete blood counts of a sample are mutually interdependent. Atypical lymphocytes and neutrophilic bands were more difficult to detect automatically.

The present data implies that the present-day strategy to reduce microscopic observation by screening all blood samples with automated white blood cell differential count is not only economically inevitable but also warranted in quality assurance. Information on the specific purpose of blood cell analysis is highly desirable in selected cases, especially with regards to atypical or abnormal lymphocytes and monocytes.

Key Words : complete blood counts (CBC), automated blood cell analyzer, flow cytometry, suspect flag, definitive flag

Introduction

The time-honored method of complete blood counts (CBC) are too much labor-demanding. One strategy is to screen blood samples by an automated leukocyte differential counting and perform microscopic observation on a relatively small proportion of them. Flow cytometric methods for this purpose have merits and demerits in common. To refer our experience on Coulter STKS[1]: 1) precision is better than the traditional microscopic observation of 200 leukocytes, especially in eosinophil, neutrophil and lymphocyte counts, 2) there is no bias in eosinophil counts, 3) there is little bias in neutrophil and lymphocyte counts unless they are interfered by morphologically abnormal cells, and 4) bias in monocyte and basophil counts tends to reflect an unique property of individual samples.

A major practical problem in flow cytometric blood cell analysis is the possibility to miss pathological cells such as blasts, immature granulocytes, abnormal and atypical lymphocytes, nucleated red blood cells (NRBC), etc. The analyzers have built-in programs to resolve this problem, which

recognize pathological and unusual cells and automatically display "suspect flags". To test the reliability of flags, we reviewed CBC data of twenty weeks duration in Yamaguchi University Hospital.

Materials and Methods

Analyzer Automated blood cell analyzer, Coulter STKS (Nikkaki, Tokyo).

Flags The warning signs for qualitative abnormalities or suspect (S) flags are built-in and their programs unknown to the users. The warning for quantitative abnormalities or definitive (D) flags can be set by the users; the selected values in our laboratory are as shown in Table 1.

Laboratory system All samples were carried by an automated sample feeding line. STKS analysis was the first, followed by preparation of smear by centrifugal spreading and automated staining. All samples with S or D flags were inspected by microscopy. Samples from Department of Hematology/Endocrinology and of Pediatrics were subjected to unconditional microscopic observation.

Table 1 Flags to signal the necessity for microscopic study

Suspect flags
Blasts, Imm grans/bands 1 or 2,
Variant lymphs.
Review slide.
NRBCs, RBC fragments,
RBC agglutination.
Platelet clumps.
Review nomogram.
Definitive flags (outliers of following ranges)
RBC 2-6 x10^{12}/L, PCV 0.1-0.54 L/L,
Hb 8-19 g/dL;
MCV 70-105 fL, MCHC 24-32 pg, MCHC 29.5-36.5 g/dL, RDW-CV 10-17%.
Platelet 50-500 x10^{9}/L, MPV 4-12 fL, PDW-CV 12-18%.
WBC 2-12 x10^{9}/L; Neutrophil 20-80% or 1-9.9 x10^{9}/L, Eosinophil ≦20%,
Basophil ≦3%, Monocyte ≦15%,
Lymphocyte 10-60% or ≦5.5 x10^{9}/L.

Data processing CBC data from Hematology/Endocrinology and Pediatrics with any of following properties on microscopic observation were extracted, grouped, and filed in StatFlex (ViewFlex, Tokyo): 1) blasts ≧1%, 2) immature granurocytes (Imms) ≧2%, 3) Imm + band form neutrophils (Bands) ≧15%, 4) atypical cell (Atyps) ≧2%, or NRBCs ≧1/100 WBC.

Results

Forty-eight percent of some 15 thousands blood samples analyzed by STKS elicited either S or D flags, or both. The CBC data of about one thousand samples were extracted and grouped according to the criteria mentioned in **Materials and Methods**. The incidence of flags in each group was tabulated.

1. Blasts

Blasts (≧1%) were found in microscopic examination of 170 blood samples from 35 patients (25 tests a patient at maximum). Sensitivity of "Blasts" and other flags for the detection of blasts were as follows (Table 2, Fig. 1). The correct flag of "Blasts" was displayed in most tests (74/78) if blasts com-

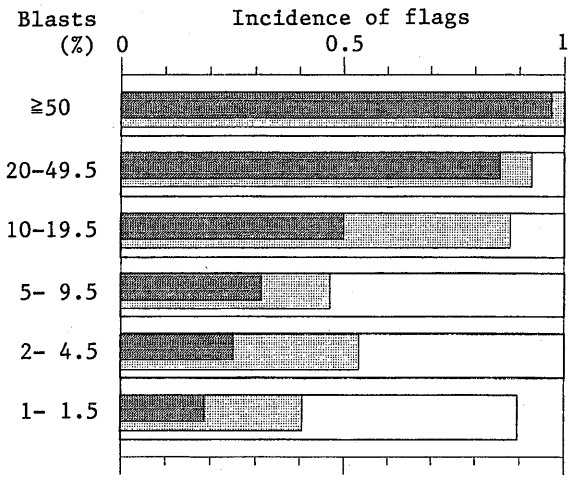

Fig. 1 Incidence of flags for blasts.
Dark-shaded column: the correct S flag of "Blasts".
Light-shaded column: any S flags for white cells, including "Review slide".
White column: any S or D flags.

Table 2 Incidence of flags for blasts

Blast (%)[1]	Number of tests	Suspect flags "Blasts"	White cell[2]	Any[3]	Definitive flags White cell[4]	Any[5]	Any flags (S or D)
≧50	64	62(0.97)[a]	64(1)	64(1)	63(0.98)	64(1)	64(1)
20-49.5	14	12(0.86)	13(0.93)	13(0.93)	13(0.93)	13(0.93)	14(1)
10-19.5	8	4(0.50)	7(0.87)	7(0.87)	8(1)	8(1)	8(1)
5- 9.5	19	6(0.32)	9(0.47)	16(0.84)	15(0.79)	17(0.90)	19(1)
2- 4.5	28	7(0.25)	15(0.54)	18(0.64)	19(0.68)	26(0.93)	28(1)
1- 1.5	37	7(0.19)	15(0.40)	19(0.51)	22(0.59)	31(0.84)	33(0.89)
≧20	78	74(0.95)	77(0.99)	77(0.99)	76(0.97)	77(0.99)	78(1)
≧10	86	78(0.91)	84(0.98)	84(0.98)	84(0.98)	85(0.99)	86(1)
≧5	105	84(0.80)	93(0.89)	100(0.95)	99(0.94)	102(0.97)	105(1)
≧2	133	91(0.68)	108(0.81)	118(0.89)	118(0.89)	128(0.96)	133(1)
≧1	170	98(0.58)	123(0.72)	137(0.81)	140(0.82)	159(0.93)	166(0.98)

1: Microscopic observation of 200 leukocytes.
2: Any S flags of "Blasts", "Imm grans/bands" 1 or 2, "Variant lymphs", or "Review slide".
3: Any of all S flags for white cells, red cells or platelets.
4: Any 'abnormal' total or differential white cell counts (cf. Table 1).
5: Any of all D flags for white cells, red cells or platelets (cf. Table 1).
a: Number and rate (in parentheses) of flag positives in each group.

prised 20% or more of the total leukocytes. The four tests in this subgroup which failed to display "Blasts" were from a patient of acute lymphoid leukemia (twice: lymphoblasts 65 and 75% which were mostly classified as lymphocytes by STKS) and another of acute myeloid leukemia (twice : monoblasts 45% which were mostly classified as neutrophils by STKS, and blasts 31% probably divided into neutrophils and lymphocytes). 2) At least one (nearly correct) S flags for leukocytes was displayed in most tests (84/86) if blasts comprised 10% or more. 3) At least one S or D flag was displayed in all tests if blasts comprised 2% or more (133/133). 4) At least one S or D flag was displayed in almost all tests (166/170) if blasts comprised 1% or more. 5) The kind of S flags tended to be incorrect (i.e. other than "Blasts") if the proportion of blasts was less than 10%. In any way almost all samples with blasts 1% or more would have been subjected to microscopic examination because of flags (Table 1) even if unconditional microscopic examination had been abandoned. Technologists could not trust the kind of flags, however; they always had to look for blasts irrespective of what kind of S or D flags were diaplayed.

Comparable results (data not shown) were obtained when only one test (the first occasion) a patient was compiled, although the number of the patients was not large enough.

Concerning the absolute number of blasts, the correct S flag was displayed in all tests (61/61) if the number of blasts was 5 $x10^9$/L or more, and in most tests (76/79) if it was 0.5 $x10^9$/L or more. The incidence of the correct S flag dropped sharply as the number of blasts became less than 0.5 $x10^9$/L. However, at least one S or D flag was displayed in almost all tests (115/116) with blasts 0.1 $x10^9$/L or more.

The flag "Blasts" was displayed by STKS in 6 tests in spite of no blasts on microscopic examination. This is probably elicited because of lymphosarcoma cells in 2 tests and reactive changes in lymphocytes in the others.

In a preliminary study which filed all CBC data of a week, "Blasts" flag was displayed for 7 times with no blasts on microscopic examination of 303 random samples, yielding an estimate of 0.98 for the specificity of "Blasts" flag.

2. Immature granulocytes and bands

Review of 236 CBC data with Imms + Bands 15% or more revealed that frequency of the correct flag "Imm grans/bands" (1 or 2) could reach only about 75% of tests even for a subgroup of samples with marked increase in young neutrophils. At least one S or D flag was displayed, if Imms + Bands comprised 25% or more. Selectivity of correct or nearly correct flags remained rather high in all subgroups.

The effects of Imm (metamyelocytes, myelocytes and progranulocytes) were studied on a subgroup consisting of 144 CBC results with 2% or more Imms and less than 15% Bands. The correct flag was displayed in most tests (19/20) if the proportion of Imms was 10% or more of the total leukocytes, while the S flags were often incorrect if it was less than 5% (Table 3, Fig. 2). At least one S or D flag was displayed (49/49) if Imms comprised 5% or more.

The effects of bands were studied on another subgroup consisting of 124 CBC results with less than 2% Imms and 15% or more Bands. The correct flag was displayed only 80% of tests (12/15) even for a group with the highest proportion of bands (30% or more) (Table 4, Fig. 3). At least one S or D flag was displayed in most tests (32/34) if Bands comprised 25% or more.

These results suggested that "Imm grans/bands" flags recognized Imms better than Bands, and that differentiation between Bands and segmented neutrophils was somewhat obscure.

3. Atypical cells

Any morphologically atypical cells other than blasts, reactive or neoplastic in nature, were classified as Atyps. They were consist-

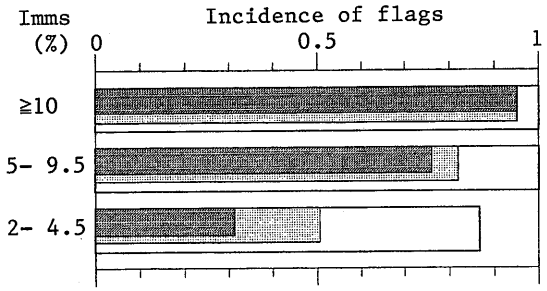

Fig. 2 Incidence of flags for immature neutrophils (progranulocytes, myelocytes and metamyelocytes). Samples with bands ≧15% have been excluded. Dark-shaded column: the correct S flag of "Imm grans" 1 or 2. Light-shaded column and white column: as in Fig. 1.

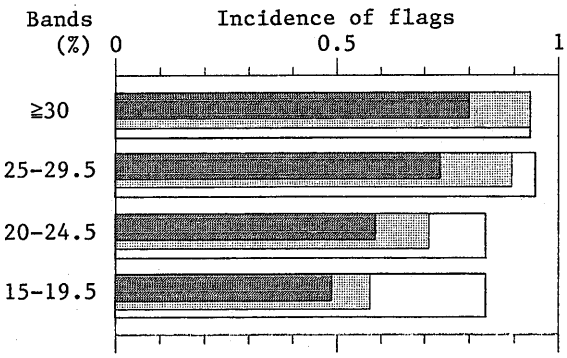

Fig. 3 Incidence of flags for neutrophilic bands. Samples with immature neutrophils ≧2% have been excluded. Columns are as in Fig. 2.

Table 3 Incidence of flags for immature granulocytes (bands <15%)

Immature (%)[1]	Number of tests	Suspect flags "Imm grans /bands" 1/2	White cell[2]	Any[3]	Definitive flags White cell[4]	Any[5]	Any flags (S or D)
≧10	20	19(0.95)[a]	19(0.95)	20(1)	15(0.75)	19(0.95)	20(1)
5-9.5	29	22(0.76)	24(0.82)	28(0.97)	18(0.62)	27(0.93)	29(1)
2-4.5	95	30(0.31)	48(0.50)	54(0.57)	73(0.77)	80(0.84)	82(0.86)
≧5	49	41(0.83)	43(0.88)	48(0.98)	33(0.67)	46(0.94)	49(1)
≧2	144	71(0.49)	91(0.63)	102(0.71)	106(0.74)	126(0.87)	131(0.91)

1-5, a: See footnotes to Table 2.

Table 4 Incidence of flags for bands (Imms <2%)

Immature (%)[1]	Number of tests	Suspect flags "Imm grans /bands" 1/2	Suspect flags White cell[2]	Suspect flags Any[3]	Definitive flags White cell[4]	Definitive flags Any[5]	Any flags (S or D)
≧30	15	12(0.80)[a]	14(0.93)	14(0.93)	12(0.80)	14(0.93)	14(0.93)
25-29.5	19	14(0.74)	17(0.89)	17(0.89)	12(0.63)	16(0.84)	18(0.95)
20-24.5	24	14(0.58)	17(0.71)	17(0.71)	15(0.62)	18(0.75)	20(0.83)
15-19.5	66	32(0.48)	38(0.58)	43(0.65)	40(0.61)	50(0.76)	55(0.83)
≧25	34	26(0.76)	31(0.91)	31(0.91)	24(0.71)	30(0.88)	32(0.94)
≧20	58	40(0.69)	48(0.83)	48(0.83)	49(0.84)	49(0.84)	52(0.90)
≧15	124	72(0.58)	86(0.69)	91(0.73)	89(0.72)	98(0.79)	107(0.86)

1-5, a: See footnotes to Table 2.

Table 5 Incidence of flags for 'reactive' lymphocytes

'Reactive' lymph (%)[1]	Number of tests	Suspect flags "Variant lymphs"	Suspect flags White cell[2]	Suspect flags Any[3]	Definitive flags White cell[4]	Definitive flags Any[5]	Any flags (S or D)
≧10	10	1(0.10)[a]	3(0.30)	7(0.70)	8(0.80)	8(0.80)	9(0.90)
5-9.5	31	6(0.19)	11(0.35)	13(0.42)	21(0.68)	22(0.71)	24(0.77)
3-4.5	78	10(0.13)	20(0.26)	22(0.28)	49(0.63)	53(0.67)	55(0.71)
2-2.5	109	9(0.08)	29(0.27)	34(0.31)	54(0.49)	64(0.59)	73(0.67)
≧5	41	7(0.17)	14(0.34)	20(0.49)	29(0.71)	30(0.73)	33(0.80)
≧3	119	17(0.14)	34(0.29)	42(0.35)	78(0.65)	83(0.70)	88(0.74)
≧2	228	26(0.11)	63(0.28)	76(0.33)	132(0.58)	147(0.64)	161(0.71)

Samples containing blasts or eliciting "Blasts" flag are excluded.
1-5, a: See footnotes to Table 2.

ed mostly of 'reactive lymphocytes' which were often associated with viral infection; the remaining cells were described as 'atypical early cells', 'lymphoma cells', 'early monocytes' and so on. A S flag of "Variant lymphs" was considered as a sign for 'reactive lymphocytes' and some of 'atypical early cells'.

Atyps comprised 2% or more of the total leukocytes in 297 tests where no blasts were found and no "Blasts" flag was elicited. The overall ineidence of "Variant lymphs" flag in this group was only 0.12 (36/297) and this figure changed little with the proportion of Atyps. Less than a half of these CBC data had at least one S flag for leukocytes. However, at least one S or D flag was displayed in almost all tests (47/48) if Atyps comprised 10% or more because of high incidence of D flags (usually for leukocytes).

The results did not differ very much if the

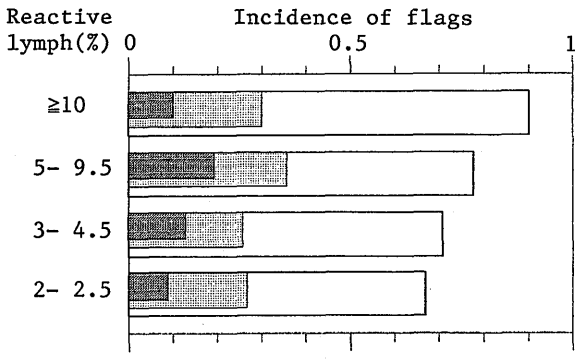

Fig. 4 Incidence of flags for 'reactive' lymphocytes. Samples containing blasts (≧1%) or eliciting the "Blasts" flag have been excluded. Dark-shaded column: the correct flag of "Variant lymphs". Light shaded column and white column: as in Fig. 1.

Table 6 Incidence of flags for atypical cells other than 'reactive' lymphocytes

Atypical cells (%)[1]	Number of tests	Suspect flags "Variant lymphs"	White cell[2]	Any[3]	Definitive flags White cell[4]	Any[5]	Any flags (S or D)
≧20	20	5(0.25)[a]	9(0.45)	13(0.65)	18(0.90)	20(1)	20(1)
10-19.5	19	2(0.10)	8(0.42)	17(0.89)	12(0.63)	18(0.95)	18(0.95)
5- 9.5	11	1(0.09)	9(0.82)	10(0.91)	2(0.18)	6(0.54)	10(0.91)
2- 4.5	15	2(0.13)	7(0.47)	7(0.47)	7(0.47)	10(0.67)	12(0.80)
≧10	39	7(0.18)	17(0.44)	30(0.77)	30(0.77)	36(0.92)	38(0.97)
≧5	50	8(0.16)	26(0.52)	40(0.80)	32(0.64)	42(0.84)	48(0.96)
≧2	65	10(0.15)	33(0.51)	47(0.72)	39(0.60)	52(0.80)	60(0.92)

Samples containing blasts or eliciting "Blasts" flag are excluded.
1-5, a: See footnotes to Table 2.

Table 7 Incidence of flags for nucleated red blood cells

NRBC/ 100WBC[1]	Number of tests	Suspect flags "NRBCs"	Red cell[2]	Any[3]	Definitive flags Red cell[4]	Any[5]	Any flags (S or D)
≧10	6	3(0.50)[a]	5(0.83)	5(0.83)	5(0.83)	5(0.83)	5(0.83)
5-9.5	12	6(0.50)	7(0.58)	11(0.92)	8(0.67)	11(0.92)	12(1)
2-4.5	40	12(0.30)	15(0.37)	30(0.75)	28(0.70)	36(0.90)	37(0.92)
1-1.5	93	19(0.20)	26(0.28)	52(0.56)	66(0.71)	87(0.93)	90(0.97)
0.5	31	3(0.10)	7(0.23)	22(0.71)	23(0.74)	28(0.90)	30(0.97)
≧5	18	9(0.50)	12(0.67)	16(0.89)	13(0.72)	16(0.89)	17(0.94)
≧2	58	21(0.36)	27(0.46)	46(0.79)	41(0.71)	52(0.90)	54(0.93)
≧1	151	40(0.27)	53(0.35)	98(0.65)	107(0.71)	139(0.92)	144(0.95)
≧0.5	182	43(0.24)	60(0.33)	120(0.66)	130(0.71)	167(0.92)	174(0.96)

2: Any S flags of "NRBCs" "RBC agglutination", "Dimorphic RBC population", or "RBC framents".
4: Any 'abnormal' data for RBC, PCV, Hb or red cell indices (cf. Table 1).
1, 3, 5, a: See footnotes to Table 1.

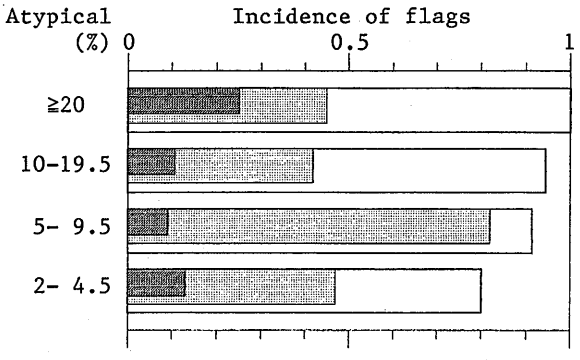

Fig. 5 Incidence of flags for atypical cells other than 'reactive' lymphocytes. Legends are as in Fig. 4.

Atyps were subdivided into 'reactive lymphocytes' and others (Table 5, 6; Fig. 4, 5), although the overall incidence of flags was higher in the latter group.

4. Nucleated red cells

The correct S flag of "NRBCs" was displayed only in a half (9/18) of CBC results (Table 7, Fig. 6), even if the number of NRBCs was 5/100WBC or more. The incidence of "NRBCs" decreased sharply with decrease in NRBC counts. However, at least one S or D flag was displayed in most tests (174/182), even at the lowest NRBC number (1/200WBC).

The S flag "NRBCs" was displayed in 92 tests where no NRBC was found by micro-

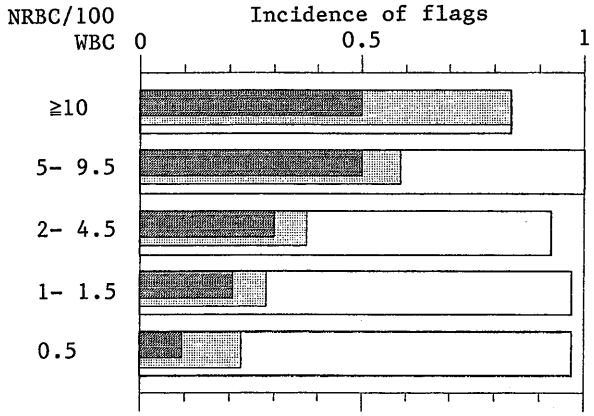

Fig. 6 Incidence of flags for nucleated red cells. Dark-shaded column: the correct S flag of "NRBCs". Light-shaded column: any S flags for red blood cells. White column: as in Fig. 1.

scopic examination. Most of them (87/92) also had other flags (e.g. S or D flags for leukocytes in as much as 81 tests) because they were abnormal in various respects.

In a preliminary study on all CBC data of a week cited above (see **1. Blasts**) two of 279 samples without NRBCs gave "NRBCs" flag. The overall specificity of "NRBCs" flag in this laboratory was therefore estimated to be 0.99, although the number of samples was too small to be statistically very reliable.

Discussion

The complete CBC including detailed microscopic observation of all blood samples is no more feasible under the today's heavy load in most clinical laboratories. Screening by automated analysis and subsequent microscopic studies on a relatively limited number of samples will partially solve the problem, and this is the only practical strategy in most hospitals.

The flag system (Table 1) would never miss untreated, typical leukemias because of monotony of leukocyte population and/or leukocytosis. A major concern is the detection of a small number of blasts, in myelodysplastic syndrome (MDS) for example. The present results indicate that the S flag "Blasts" is not sensitive enough by itself for this purpose. However, blood samples containing blasts strongly tend to have other abnormalities, too, and almost always they are somehow segregated from nearly normal samples. This is because abnormal CBC data for a single blood sample are mutually interdependent. Unconditional microscopic examination of all blood samples because of fear of missing blasts does not seem to be warranted in an extremely busy laboratory. The same appears to be the case for Imms.

The performance of flags for Bands may pose some dispute. STKS seems to discriminate between Bands and segmented neutrophils rather incompletely. Moreover, Bands could be increased under normal total and differential leukocyte counts. Accordingly some 20% of cases with increased Bands (≧ 15%) may easily be missed by the present flag system. Meanwhile, the necessity for differentiation between Bands and segmented neutrophils is questioned[2], the criteria of left shift as well. Serum C-reactive protein (CRP) would be a far better indicator of inflammation than the proportion of Bands.

"Variant lymphs" flag is too insensitive to be of practical value. This is understandable because criteria for atypical lymphocytes are rather equivocal and subjective. This is true especially for 'reactive lymphocytes' Atypical cells other than 'reactive lymphoeytes' usually accompany other abnormalities, and their detection is similar to that of blasts: at least one S or D flag is displayed although it often bears an incorrect sign.

The identification of NRBCs is quite certain in microscopic observation, yet they are easily missed by STKS. However, they almost always accompany other abnormalities, eliciting at least one S or D flag.

Flags are far from perfect concerning the correctness of S flags. They are rather sensitive, however, in segregating samples to be studied microscopically, while some difficulties are posed by atypical lymphocytes and Bands. It is almost certain, although no data have been presented, that the system is unable to warn about such morphological details as heavy granules, inclusion bodies, parasites and so on. The specific purpose of CBC should be informed to the laboratory in some particular cases.

Conclusion

The suspect flags are not so sensitive and specific by themselves as to be trusted as descriptive of the properties of the blood sample, e.g. increase in blasts, immature and band form neutrophils, atypical lymphocytes or nucleated red cells. However, the multitude of suspect and definitive flags as a whole can somehow segregate most blood samples to be studied by microscopic examination, especially those containing increased number of blasts or nucleated red cells. The flags are less efficient for the detection of immature and band form neutrophils. They are not very reliable in recognizing 'reactive lymphocytes'.

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