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Short communication

Comparative study of the efficacy of Wright-Giemsa stain and Liu's stain in the detection of Auer rods in acute promyelocytic leukemia

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ABSTRACT

In view of the importance of Auer rods in the rapid diagnosis of acute promyelocytic leukemia, we compared the results of Wright-Giemsa stain and Liu's stain (a rapid and simple stain, which is also a kind of modified Romanowsky stain) in the detection of Auer rods. This study was based on 53 cases of acute promyelocytic leukemia. Two staining methods were respectively performed on the bone marrow smears of these cases, and presence of Auer rods as well as nuclear features, cytoplasmic features and the degree of granularity of the cytoplasm were compared in each case. Our results showed that the occurrence of Auer rods as well as faggots in leukemic promyelocytes were significantly higher under Liu's stain than under Wright-Giemsa stain. Significant differences also existed in the occurrence of hypergranular cells and cytoplasmic protrusions between smears stained with Liu's stain and Wright-Giemsa stain. Liu's stain is important for the rapid diagnosis of suspicious APL, especially in recognizing Auer rods.

Introduction

Acute promyelocytic leukemia (APL), M3 subtype of acute myeloid leukemia (AML) was first recognized for its particularly poor clinical outcome due to a serious hemorrhagic syndrome, which is related to disseminated intravascular coagulopathy and abnormal fibrinolysis (Hillestad, 1957; Stone and Mayer, 1990). It was further characterized by an exquisite sensitivity to all-trans retinoic acid (ATRA) (Barbui et al., 1998; Frankel et al., 1994). In 1976, the French-American-British (FAB) cooperative group revised the morphological description of APL with recognition of two main morphological subtypes: (a) classical hypergranular promyelocytic leukemia (M3) and (b) hypogranular or microgranular promyelocytic leukemia variant (M3v) (Bennett et al., 1980). The former is characterized by abundant brightly granules plus intracellular inclusions (Auer rods), usually in bundles (faggots). The latter variant, accounting for 15–20% of APL cases, has a characteristic bilobed nucleus without granules or containing only a few fine azurophilic granules with usual staining; however, at least a few cells with all the cytoplasmic features of M3 are present. Auer rods are often present and may be in bundles. Another rare basophilic morphological variant of AML-M3 contains immature basophilic cells with cytoplasmic projections (Castoldi et al., 1994; Avvisati et al., 2001). Cells with Auer rods and cells containing multiple Auer rods or faggot cells are also present in leukemic cells of the rare subtypes of AML-M3 (Behm, 1999). Thus, the diagnostic Auer rods found in leukemic cells can provide an important basis for the diagnosis of APL.

The rapid and accurate recognition of APL is very important because of the need for urgent specific treatment to avert the risk of life-threatening hemorrhage (Gillis and Blaszkowsky, 1995; Visani et al., 2000). Whereas immunophenotype, cytogenetic and molecular studies allow a precise diagnosis of APL (Sucic et al., 2002), however, they are very time consuming. Because initiation of therapy is highly dependent upon the successful morphological diagnosis, this study sought to evaluate the recognition of Auer rods in leukemic promyelocytes using Liu's stain (a modified Romanowsky stain, which is widely used in China as a quick stain in bone marrow morphology as well as a simple stain in exfoliative cytology) compared with Wright-Giemsa stain in 53 APL cases.

Materials and methods

All 53 patients enrolled in the study were diagnosed as APL in the Wuhan Union Hospital from October 2009 to December

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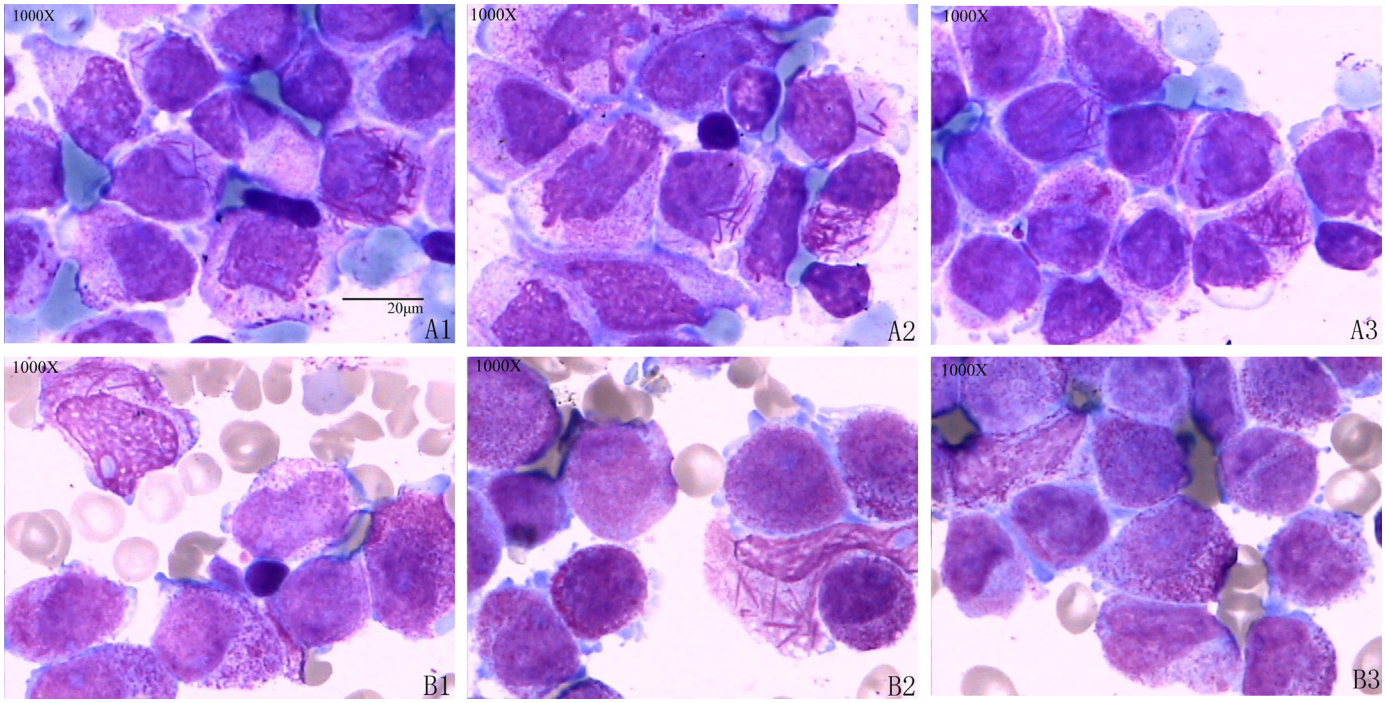

Fig. 1. Comparison of Liu's stain and Wright-Giemsa's stain in a case of APL. (A1–A3) Bone marrow smears stained with Liu Solution. Red cells were gray-blue, showing deeper color compared with Wright-Giemsa's Stain. More cells with multiple Auer rods and fewer hypergranular cells were seen. The proportions of cells with Auer rods, cells with multiple Auer rods, hypergranular cells, cells with cytoplasmic protrusions, bilobed variant cells were 12%, 12%, 32%, 40% and 5%, respectively. (B1–B3) Bone marrow smears stained with Wright-Giemsa's stain. Red cells were stained pink. Few cells with multiple Auer rods and more hypergranular cells were seen. The proportions of cells with Auer rods, cells with multiple Auer rods, hypergranular cells, cells with cytoplasmic protrusions, bilobed variant cells were 4%, 3%, 73%, 48% and 6%, respectively.

2011. Peripheral blood test, bone marrow aspirate, flow cytometry immunophenotyping, cytogenetic analysis and molecular analysis were required for the diagnosis. Informed consent was obtained from all patients before the study, which received approval from the Ethical Committee of the Tongji Medical College, Wuhan, China.

All bone marrow aspirates stained by both Liu Solution (Group A) and Wright-Giemsa's Stain (Group B). Taiwan Baso Company provided both Wright-Giemsa's Stain and Liu Solution, including Liu A Solution and Liu B Solution. Wright-Giemsa stain was used according to standard stains described in the literature (Dunning and Safo, 2011).

Liu's staining was performed according to the following staining methods. Liu A Solution was added to air-dried bone marrow smears and allowed to stand for 30 s. Then without discarding the Liu A Solution, Liu B Solution (about twice the dosage of A) was added to the smears. The two solutions were mixed thoroughly to stain for 1 min. Then the smears were washed with tap water and air dried or with filter paper prior to microscopic examination.

Bone marrow smears stained with both Wright-Giemsa's Stain and Liu Solution from all cases were independently evaluated by two hematopathologists. Each case had a minimum of 20 fields from bone marrow smears registered at a 1000-fold magnification, and, according to general agreement, was classified by FAB criteria (Bennett et al., 1976). Furthermore, 200 leukemic promyelocytes in each case were examined for nuclear features (regular or irregular, especially bilobed), cytoplasmic features, the degree of granularity of the cytoplasm (hypergranular or hypogranular) and presence of Auer rods, scanned smears for faggots. Faggots were defined by the presence of at least three Auer rods per cell.

Statistically significant differences were compared between the two kinds of stain methods by paired *t*-test. The analysis performed used SPSS ver.15.0 statistical software (SPSS, IBM, Chicago, IL, USA). Two-sided *P*-values were used throughout. *P*-values were considered significant when <0.05.

Results

All 53 APL cases showed overexpression of CD13, CD33 and MPO. Lack or decreased CD34 expression, lack of HLA-DR and expression of CD2 were detected in 49 cases (92.5%), 43 cases (81.1%) and 13 cases (24.5%) respectively. The classical *t* (15; 17) (q22; q12) translocation accounted for 51 cases (96.2%), with 47 cases with single *t* (15; 17) and 4 other cases accompanied by del(7), del(17p), del(1q), and del(9) respectively. We detected the reciprocal PML/RARα gene in all 51 patients with *t* (15; 17). Two other cases presented with the *t* (11; 17) (q23; q21) translocation and PLZF/RARa gene.

Auer rods were seen in each of the 53 M3 cases with both Liu Solution and Wright-Giemsa's stain; faggots were absent in four cases with Wright-Giemsa's stain and in 2 cases with Liu Solution. The mean proportion of cells with Auer rods was 7.70% with Liu Solution as compared with 5.58% with Wright-Giemsa's stain ($t = 2.173$, $P < 0.05$) and the mean proportion of faggots was 6.25% under Liu Solution as compared with 2.70% under Wright-Giemsa's stain ($t = 8.543$, $P < 0.05$) (Fig. 1). Significant differences were also present in the occurrence of hypergranular cells and cytoplasmic protrusions between bone marrow films stained with Liu's stain and Wright-Giemsa stain (Table 1; Fig. 1). There were no significant differences in the rates of occurrence of bilobed variant cells between the two staining methods (Table 1).

Discussion

Despite the impressive improvement in the fields of molecular biology and cytogenetics, cell morphology of bone marrow and peripheral blood remains the diagnosis cornerstone to identify the various subtypes of hematopoietic neoplasms, especially