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Negative stain for myeloid peroxidase and Sudan black B in acute promyelocytic leukemia (APL) cells: report of two patients with APL variant

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We report two patients with APL variant with negative stains for MPO and SBB, although CE was positive. This finding highlights the improtance of CE in diagnosis of APL with microgranular variant.

Approximately 25% of patients with acute promyelocytic leukemia (APL) have a microgranular variant (M3v), characterized by marked leukocytosis, severe coagulopathy, and intensely staining for myeloid peroxidase (MPO), Sudan black B (SBB), and chcoroacetate esterase (CE). We report here two APL M3v patients with negative stains for MPO and SBB in their leukemic promyelocytes.

Case Reports. *Patient #1.* A 32-year-old Chinese woman was referred to our hospital with a 15-day history of ecchymoses. Physical examination revealed large ecchymoses of 4-6 cm in diameters on her lower extremities, slight tenderness of sternum and epigastria, and hepatomegaly of 1cm below right costal margin. Blood cell count showed: Hb 90 g/L, WBC $46.7x10^9$/L with 76% micro-granular promyelocytes, and PLT $41x10^9$/L. The thrombin time (TT), prothrombin time (PT) and activated partial thrombin time (APTT) exceeded normal controls by 12.7 seconds, 20 seconds and 13 seconds respectively, plasma fibrinogen level was 0.33 g/L and plasma protamine paracoagulation (3p) test was positive. The bone marrow was hypercellular with 4.0% myeloblast and 90.0% microgranular promyelocytes. The positivity rates of MPO and SBB staining in bone marrow cells were 18% and 8%, respectively, and CE staining was positive in 98% of cells with 70% of them staining strongly (Figure 1). Cytogenetic and RT-PCR studies demonstrated the characteristic reciprocal translocation of t(15;17) (q22;q21) and PML/RARalpha transcript, respectively. Phenotyping analysis revealed CD34 14%, CD3398%, CD1341%, CD1535%, CD320%. The patient was treated with all trans retinoic acid (ATRA) and heparin, but died three days later of acute renal failure.

Patient #2. A 38-year-old Chinese lady was referred with a complaint of weakness, gum bleeding and menorrhagia for ten days. Physical examination revealed pallor, petechiae on skin and slight tenderness of the sternum. Blood cells count showed Hb 51g/L, WBC $70.5x10^9$/L with 87% microgranular promyelocytes, and PLT $17x10^9$/L. TT and PT exceeded normal controls by 11.2 and 13.5-seconds, respectively, plasma fibrinogen level was 35 g/L and the 3P test was positive¨D-dimer and fibrin degradation products exceeded 0.5 μg/L and 5.0 μg/L respectively. Bone marrow was hypercellular with 30.5% myeloblasts and 64.0% microgranular promyelocytes. MPO and SBB stains were negative, while the positive rate of CE staining was 72% among which only 6% of cell were strongly positive (Figure 2). The patient was treated with ATRA and achieved complete remission on day 56 of treatment. The patient refused to receive consolidation chemotherapy, relapsed and died on day 281 after diagnosis.

Strong MPO, SBB, and CE positivities are the cytochemical characteristics of APL (M3 or M3v).[1] Studies of biosynthesis and processing of MPO in a human APL cell line found that primary translated MPO precursor with MW 89,000 had no detectable MPO activity, but further modification of MPO at lysosomes activated MPO.[2] This suggested that defective co-translational/post-translational processing of a MPO precursor could result in production of

an aberrant form of MPO not packaged in the azurophilic granules during the promyelocytic stage of development.[2] Derived from HL60 cell line by growth with actinomycin D, the HL60-A7 cell line lost sudanophilia and contained large azurophilic granules deficient in MPO.[3] The products of HL60-A7 RNA translation contained less than 5% of immunoreactive MPO found in HL60 cell *in vitro.* The low number but strongly-staining cells in HL60-A7 and patient #1 suggested that the MPO gene was not totally deleted but might be partially transcribed or gave rise to an unstable inefficient RNA. Pui *et al.* reported that low levels of MPO reactivity distinguished cases of childhood acute non-lymphoblastic leukemia in which the blast cells co-expressed lymphoid and myeloid markers.[4] This was the case in patient #1 who expressed CD3 on cells. Tomonaga *et al* also found a 25-year-old female case with negative staining for MPO, who showed a fulminant course with extensive DIC.[5] Our observations and those of others, indicate that the cytochemical pattern of APL is heterogeneous; the negative stains for MPO and SBB could be found in a subset of young female patients with evident leukocytosis and coagulopathy. The cells might co-express lymphoid and myeloid markers. It is worth drawing attention to observations that CE positivity is present when MPO activity and SBB are absent in these patients.

References

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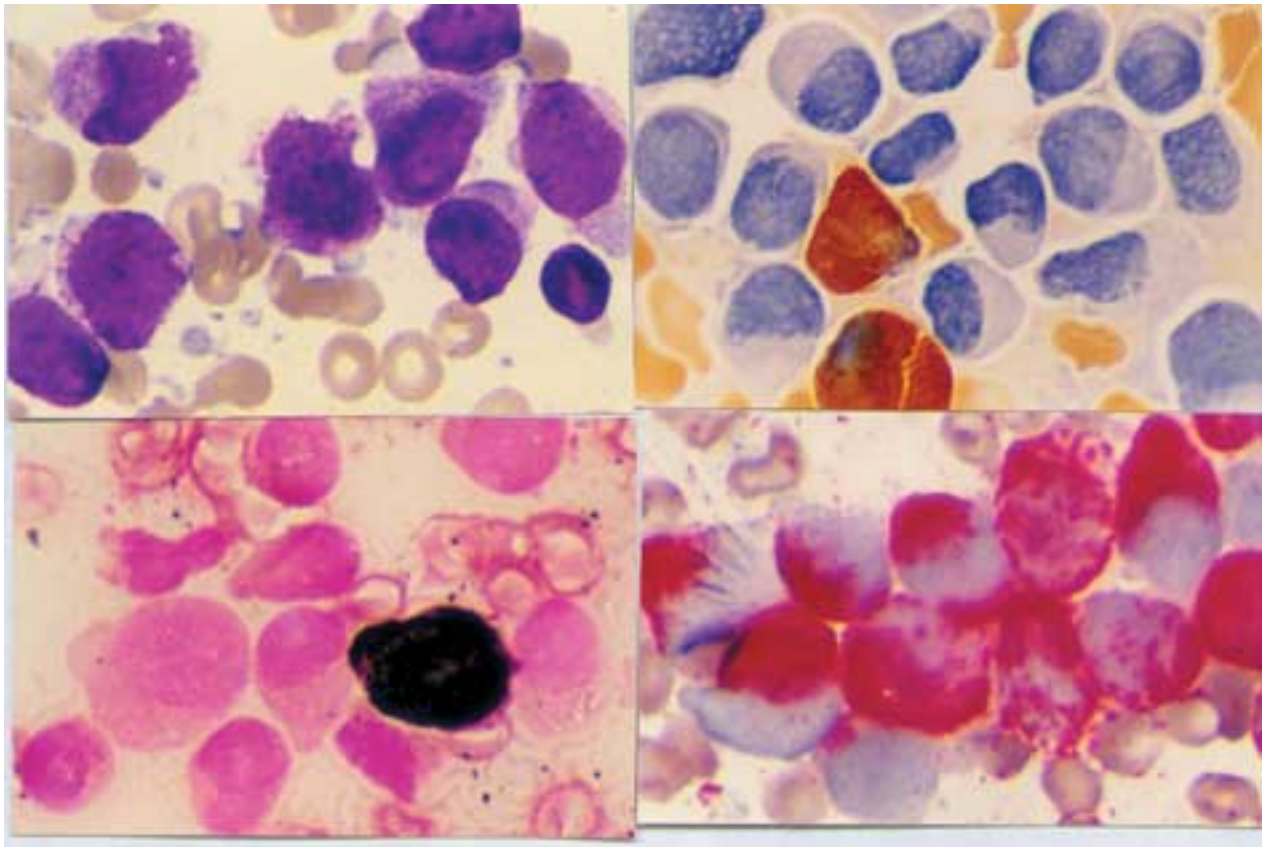

Fig 1.Morphology and cytochemical stains of the patient 1 (magnification ×1,000).

A: myeloblasts and micrograniule promyelocyte were 4.0%, 90.0% respectively, with no Auer rod. [May-Grunwald-Giemsa (MG) stain]; B: 18% of cells were strongly MPO positive, but the remaining 82% were completely negative; C: 8% of cells were strongly SBB positive, but the remaining 92% were completely negative; D: 98% of cells showed modest to strong CE activity of diffuse cytoplasmic pattern.

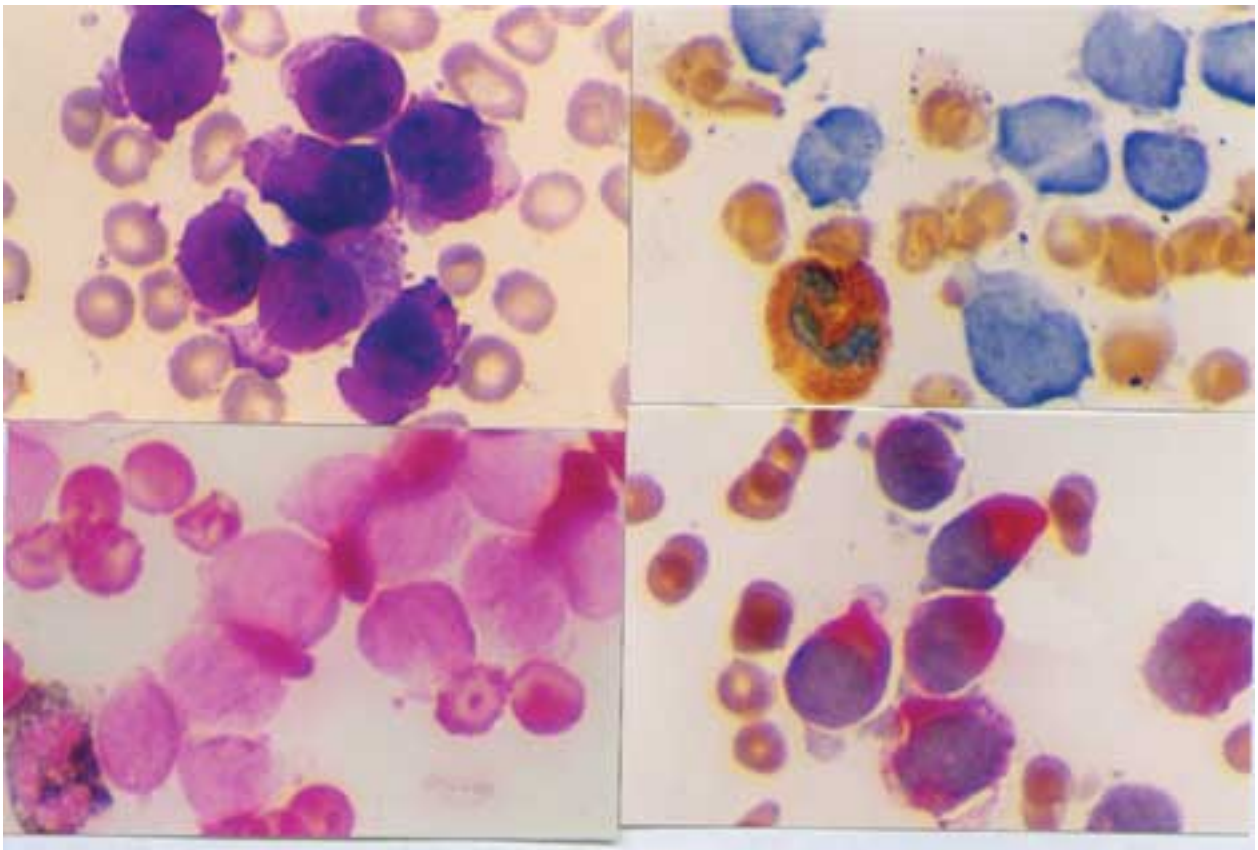

Fig 2. Morphology and cytochemical stains of the patient 2 (magnification ×1,000).

A: myeloblasts and microgranule promyelocyte were 30.5%, 64.0% respectively, with no Auer rod (MG stain); B: All of cells were MPO negative; C: All of cells were SBB negative; D: 72% of cells showed positive CE staining, in which only 6% cell were strongly positive.