results indicate that the cut-off level (≥10 %) of significant dysE according to the WHO classification including RCACC may not be suitable. ITP or HA patients with original WHO dysE ≥10 % may be misdiagnosed as having MDS. The misdiagnosis of MDS is a serious problem. Naturally, these patients do not respond to drug therapies such as azacitidine. Stem cell transplantation may become a candidate second-line treatment for these patients.

The distinction of MDS and the HA is relatively easy by laboratory findings. On the other hand, distinction of MDS and ITP may not be easy. Most of our MDS patients showed original WHO dysE ≥20 %. In contrast, most of ITP/HA group did not show original WHO dysE ≥20 %. The MDS patients without original WHO dysE ≥20 % had dysG and/or dysMgk ≥10 % whereas none of the ITP or HA patients had WHO dysG and/or dysMgk ≥10 %. For suitable criteria of dysE for the diagnosis of MDS, we think that raising the threshold from 10 to 20 or 30 % in original WHO dysE may be appropriate. In our study, the percentage of MDS patients without strict dysE ≥10 % was not particularly low (29 %). In contrast, the percentage of ITP and HA patients with strict dysE ≥5 % was low (14 and 8 %, respectively). None of the ITP or HA patients showed strict dysE ≥10 %. And none of the ITP or HA patients showed ring sideroblasts. The criteria of strict dysE ≥10 % and/or ring sideroblasts were helpful for identifying non-malignant conditions. If RCACC is not included in dysE, strict dysE of the present study, the threshold of dysE should be 10 %.

RCACC is identified by light microscopic examination of WG- or MG-stained films. The dysplastic form is characterized by the abnormal clumping of chromatin. The assessment of RCACC was the most difficult in dysE, and therefore, the concordance rate was insufficient. In the results of present study, the possibility that non-clonal disorders were misdiagnosed as MDS was suggested. We think that concordance rate of RCACC by the observers may be poor. Therefore, in the method of present study, when at least one of the two hematologists judged RCACC, we decided to judge the cell as RCACC. We think that use of this method may raise the ratio of RCACC. The suitable criteria for dysE are different whether RCACC is included in dysE. If RCACC is included in dysE criteria, threshold of dysE should be raised from 10 %. On the contrary, if RCACC is not included in dysE criteria, we think that '10 %' is the suitable threshold of dysE.

To improve the cytomorphologic problem of RCACC, we are performing a quantitative analysis of chromatin clumping between RCACC and normal erythroblasts by a modification of Kerr's method [19]. We believe that the quantitative evaluation method is useful for the diagnosis of MDS.

In summary, if RCACC is included in dysE criteria, raising the threshold from 10 to 20 or 30 % in dysE including RCACC (the original WHO dysE) may provide more suitable criteria in the diagnosis of MDS. In another method, if RCACC is not included in dysE, strict dysE of the present study, threshold of dysE should be 10 %. The original WHO dysE ≥20 or 30 % or strict dysE ≥10 % may reduce the risk that a non-clonal disease is misdiagnosed as MDS.

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Compliance with ethical standards

Conflict of interest The authors have no potential conflict of interest to report.

References

1. Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision-making. J Clin Oncol. 2005;23(30):7594–603.
2. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51(2):189–99.
3. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organization classification of tumours. Pathology and genetics. Tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001.
4. Swerdlow SH, Campo E, Harris NL, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008.
5. Wickramasinghe SN. Diagnosis of megaloblastic anaemias. Blood Rev. 2006;20(6):299–318.
6. Kaushansky K, Lichtman M, Beutler E, Kipps K, Prchal J, Seligsohn U, editors. Williams hematology. 8th ed. New York: McGraw-Hill Professional; 2010.
7. Jimenez-Balderas FJ, Morales-Polanco MR, Gutierrez L. Acute sideroblastic anemia in active systemic lupus erythematosus. Lupus. 1994;3(3):157–9.
8. Carpenter SL, Zimmerman SA, Ware RE. Acute parvovirus B19 infection mimicking congenital dyserythropoietic anemia. J Pediatr Hematol Oncol. 2004;26(2):133–5.
9. Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, British Committee for Standards in Haematology, et al. Guidelines for the diagnosis and management of acquired aplastic anaemia. Br J Haematol. 2009;147(1):43–70.
10. Vardiman JW. Hematopathological concepts and controversies in the diagnosis and classification of myelodysplastic syndromes. Hematol Am Soc Hematol Educ Program. 2006;2006:199–204.
11. Bain BJ. The bone marrow aspirate of healthy subjects. Br J Haematol. 1996;94(1):206–9.
12. Parmentier S, Schetelig J, Lorenz K, Kramer M, Ireland R, Schuler U, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. Haematologica. 2012;97(5):723–30.

13. Ramos F, Fernandez-Ferrero S, Suarez D, Barbon M, Rodriguez JA, Gil S, et al. Myelodysplastic syndrome: a search for minimal diagnostic criteria. Leuk Res. 1999;23(3):283–90.
14. Matsuda A, Germing U, Jinnai I, Iwanaga M, Misumi M, Kuendgen A, et al. Improvement of criteria for refractory cytopenia with multilineage dysplasia according to the WHO classification based on prognostic significance of morphological features in patients with refractory anemia according to the FAB classification. Leukemia. 2007;21(4):678–86.
15. Maslak P. Refractory Anemia with excess Blasts-2. ASH Image Bank. http://imagebank.hematology.org/Default.aspx. Accessed 15 Aug 2015.
16. Vardiman JW. Myelodysplastic Syndrome: Refractory Anemia with Ringed Sideroblasts (RARS)-5. http://imagebank.hematology.org/Default.aspx. Accessed 15 Aug 2015.
17. Della Porta MG, Travaglino E, Boveri E, Ponzoni M, Malcovati L, et al. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. Leukemia. 2015;29(1):66–75.
18. Bennett JM. Morphological classification of the myelodysplastic syndromes: how much more education of diagnosticians is necessary? Haematologica. 2013;98(4):490–1.
19. Kerr E, Kiyuna T, Boyle S, Saito A, Thomas JS, Bickmore WA. Changes in chromatin structure during processing of wax-embedded tissue sections. Chromosome Res. 2010;18(6):677–88.