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Clinical Image
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Input of Next Generation Sequencing for the Diagnosis of an Uncommon Anemia

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Clinical Image

A 57 year-old man was referred for investigation of anemia in the context of minor beta-thalassemia proved by electrophoresis of hemoglobin, dilated cardiomyopathy with aortic ectasia, asthma, hiatal hernia and cholecystectomy. Anemia was already known due to thalassemia with hemoglobin level around 100 to 95g/l level, mean corpuscular volume around 58 fl and hemoglobin electrophoresis with an excess of Hemoglobin A2 and F proving the minor beta thalassemia. But, since few months, hemoglobin was falling dramatically to 84g/l. A first bone marrow examination was performed and showed dysplastic features (basophilic stippling, laminated cytoplasm) only on erythroid lineage (Figure 1A) but involving less than 10% of the lineage, and the presence of 48% ringed sideroblasts (Figure 1B). Nevertheless those erythroid dysplastic features could be attributed to thalassemia as well as myelodysplastic syndromes due to the increased erythropoiesis observed in thalassemia. Ringed sideroblasts are not specific of dysplastic syndrome. Ringed sideroblasts can be seen in some specific deficiencies (zinc, vitamin B6) [1,2] or intoxications (alcohol, lead, benzene, isoniazid, copper) [3,4]. All these etiologies were eliminated by questioning and biological blood tests. EPO level was 20.4 U/l and ferritin level was normal (389ng/ml). The patient received no prior treatment (neither transfusion, nor erythropoietin stimulating agents (ESA)) and was referred for a secondary opinion to our university hospital center. At this time, 3 months later after the first bone marrow examination, a new bone marrow aspiration was performed with a flow cytometry, karyotype and molecular analyses, and a bone marrow biopsy. Bone marrow aspiration results were similar to the first one with abnormalities of the erythroid lineage: laminated and granular cytoplasm and abnormalities of karyorrhexis, but ringed sideroblasts were not found (Figure 1C). Moreover, the flow cytometry Ogata score was low (score=1/4) [5] and the karyotype were normal. There were some dystrophic megakaryocytes and micromegakaryocytes in the marrow biopsy without fibrosis (Figure 1D). At this stage, it was difficult to decide between myelodysplastic syndrome or increased erythropoiesis due to thalassemia. Finally, the molecular biology analysis using a next generation of sequencing assay by using the Ion AmpliSeq™ library kit 2 (Life technologies), found mutations concerning genes that are mostly involved in myelodysplastic syndromes. Deep sequencing identified 3 candidate somatic mutations: a missense in SETBP1 (I871T) and two ins/del mutation inducing a premature stop codon in SRSF2 and TET2. All these mutations were previously described as somatic [6-8] and were confirmed by direct sequencing. Moreover, the variant allele frequency for each mutation was 45%, 43% and 41% respectively for TET2, SRSF2 and SETBP1, corresponding probably to one major clone. Concerning the ringed sideroblasts, the SF3B1 mutation which is present in 60-80% of the refractory anemia with ringed sideroblasts (RARS) [9], was not found in our patient, leading us to think that the ringed sideroblasts were not related to the typical MDS subtype RARS. Finally, the diagnosis of undetermined myelodysplastic syndrome (MDS-U) was retained thanks to the presence of an array of arguments: the association of the three mutated genes in a relatively young patient and with the presence of SRSF2 and SETBP1 mutations, which are not so commonly found in "control patients without any cytopenia" [10,11], and the dystrophic megakaryocytes in bone marrow biopsy which are not found in thalassemia. A treatment with erythropoietin stimulating agents (ESA) was begun. Three months after the beginning of ESA, Hb level increased from 86g/l to 108g/l which corresponds to a hematological erythroid response according to IWG 2006 criteria [12].

This case illustrates the aid available from molecular biology analyses in situations where the

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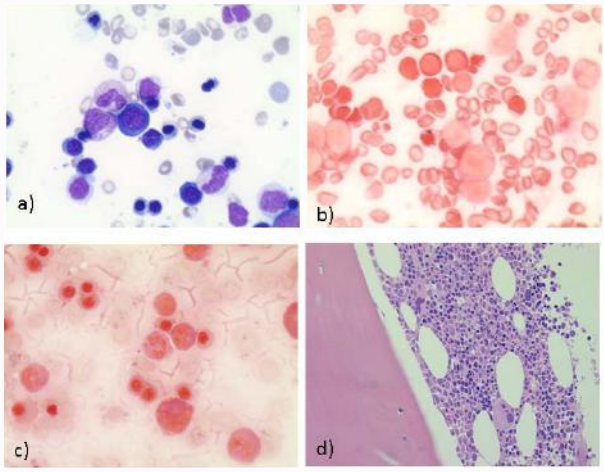

Figure 1: a) May-Grunwald-Giemsa (MGG) stained bone marrow aspiration, first marrow examination, X400; b) Perls stained bone marrow aspriation, first marrow examination, X400; c) Perls stained bone marrow aspiration, second marrow examination, X400; d) Hemalin-Erythrosine-Safran (HES) stained bone marrow trephine biopsy, X40.

diagnosis of myelodysplastic syndrome is not formally raised by the morphological study of the bone marrow aspiration. Moreover, in this case, the percentage of morphological abnormalities was below the percentage required for the diagnosis of myelodysplastic syndrome and cytogenetic analyses were normal, evoking a new kind of myelodysplastic syndrome based on a molecular biology classification [13].

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