INTRODUCTION

Myelodysplastic syndrome (MDS) is a clonal hematopoietic neoplasm characterized by ineffective hematopoiesis. However, vague pathogenesis, various phenotypes, and genetic mutations make it difficult to diagnose this disease. Usually, patients with MDS show pancytopenia in their peripheral blood. Currently, there is no definite marker to distinguish MDS from other hematologic diseases based on the patient’s peripheral blood. Findings of dysplastic features from peripheral blood are limited. Large or abnormally granulated platelets, pseudo–Pelger-Huët anomaly and hypogranular cytoplasm in neutrophils, basophilic stippling, and poikilocytosis in red blood cells (RBCs) are features that can be observed in a peripheral blood smear using light microscopy [1]. However, these features are not unique to MDS and are not observed frequently. Bone marrow examination must therefore be performed to diagnose MDS.

In this study, we employed optical diffraction tomography (ODT) to perform three-dimensional (3-D) imaging of RBCs from a patient with MDS. Based on laser interferometry, ODT reconstructs the 3-D refractive index (RI) distributions of samples without a labeling procedure [2]. The RI tomograms of individual RBCs provide information about the morphological, biochemical, and mechanical parameters at the individual cell level [3]. Conventionally, 3-D images of RBCs have been obtained using scanning electron microscopy (SEM) [4]. However, SEM has limitations in its time-
consuming sample preparation procedures as well as high cost. In contrast, ODT provides rapid and simple 3-D live-cell imaging.

Currently, Wright-staining of peripheral blood and observation with light microscopy is the standard method to examine blood cell morphologies. However, this technique can only provide the projected 2-D images of cells. Because MDS features in peripheral blood cells are non-specific and difficult to detect by conventional protocols, we investigated new characteristics of MDS RBCs in peripheral blood by examining the 3-D RI tomograms of individual RBCs. Using ODT, we found distinct cup-like-shaped RBCs in a patient diagnosed with MDS.

CASE REPORT

The patient is a 46-year-old male who was diagnosed with MDS with excess blasts-2 in July 2017. His karyotype was normal. He underwent an allogeneic hematopoietic stem cell transplantation (HSCT) in January 2018. He visited our hospital for regular follow-ups and underwent bone marrow and peripheral blood examinations on May 14, 2018. The bone marrow showed normocellular marrow showing trilineage regeneration with little remains of erythroid dysplastic features. The blasts count was 0.2% and had 4% of dyserythropoietic features such as multinuclearity. Post-HSCT chimerism monitoring of peripheral blood showed no signs of recipient chimerism. On determining his complete blood count, the hemoglobin level was found to be 7.8 g/dL; platelet count, 57 × 10^9/L; white blood cell count, 4.6 × 10^9/L; and mean corpuscular volume, 106.5 fL. The blood smear demonstrated pancytopenia. He had occasionally been receiving leukoreduced RBCs to correct severe anemia since the HSCT. The last RBC transfusion before performing the bone marrow examination was conducted one month ago. The RBCs showed anisopoikilocytosis with hypochromic and macrocytic features (Fig. 1A). Few stomatocytes were observed, but their proportion was not clinically significant (<5%). With the same peripheral blood, we reconstructed 3-D RBCs using an ODT setup microscope. For the normal reference, cells from a healthy individual who visited our hospital for a regular health check-up were used. The 3-D RBC acquisitions from the patient and the healthy individual were performed on the patient’s next out-patient visit on May 29, 2018.

We collected blood samples in the EDTA anticoagulant tube which were left over from the complete blood count tests performed by the Department of Laboratory Medicine, Asan Medical Center. Collected blood (5 μL) was diluted with Dulbecco’s phosphate-buffered saline (DPBS) without calcium and magnesium (1 mL) (Thermo Fisher, Waltham, MA, USA). The diluted blood was loaded on a coverslip of 25 × 50 mm (C025501, MATSUNAMI GLASS Ind., LTD., Japan) and covered with another similar coverslip. 3-D RI tomograms were measured using a commercial ODT setup microscope, HT-1H (Tomocube Inc., Daejeon, Korea). This system re-
constructions the 3-D RI tomogram of a sample from multiple 2-D holographic images of the sample obtained using various illumination angles. For the visualization and analysis of the measured 3-D RI tomograms and for obtaining RBC parameters, a commercial software (Tomostudio, Tomocube Inc., Daejeon, Korea) was used. The sample prepared with coverslips was placed between the condenser and objective lens. RBCs that were sedimented on to the coverslip were randomly selected from the 2-D hologram view. Multiple RI tomograms were recorded for each RBC and reconstructed into a 3-D image. Forty to 60 RBCs were reconstructed into 3-D images per sample [2, 5]. The study was conducted according to the principles of the Declaration of Helsinki, using remaining blood; the procedures described above were approved by the responsible ethics committee of Asan Medical Center (IRB project number: 2018-0071, 2018-0072).

There were unique cup-shaped RBCs in the patient’s peripheral blood (Fig 1B). The 3-D reconstructed RBCs were divided into 3 groups based on their morphology: cup-shaped, abnormal shapes other than the cup-shape, and normal (Fig. 1B-D). We found that 37.5% and 32.8% of RBCs from the MDS patient showed cup-shaped RBCs whereas none were found in the control (Table 1). Results from light microscopy were unable to differentiate between cup-shaped and normal RBCs.

<table>
<thead>
<tr>
<th>Table 1. RBC counts by morphology in each sample</th>
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*Cup-like shaped RBCs are not included in this category.
Abbreviations: RBC, red blood cell; MDS, myelodysplastic syndrome.

DISCUSSION

Easy acquisition of 3-D images of RBCs is a great advantage of ODT microscopy. It is not possible to discover unique morphologic characteristics during routine peripheral blood smear examinations which cover only the surface of RBCs. The only clinically significant findings were pancytopenia with increased mean corpuscular volume (MCV), and red blood cell distribution width (RDW) observed in the patient’s Wright-stained peripheral blood slide. The patient had undergone HSCT which was apparently successful. However, pancytopenia in the peripheral blood and signs of dysgranulopoiesis in the bone marrow suggested that MDS was not fully cured. A high number of poikilocytes including spherocytes and stomatocytes demonstrates changes in cell volume or membrane surface area due to changes in the composition of membrane and cytoskeletal elements [6, 7]. It is possible that cup-shaped RBCs are one of the dyserythropoietic features that can be observed in peripheral blood. The RBC membrane consists of a lipid bilayer and spectrin network. The spectrin network confers resistance to bending force and shear strain, and maintains the shape of the RBC. It is tightly attached to the lipid bilayer in normal RBCs under normal conditions. This tight and elastic structure can become dissociated in pathologic conditions [8]. The integrity of RBC structure is determined by various properties such as membrane composition, cytoplasm viscosity, spectrin network, shape, and size [9, 10]. Genetic alteration also plays a major role in the formation of dysplastic RBCs. For example, 5q deletion affects the ribosomal protein RPS14, leading to abnormal erythrocyte maturation. Many mutations have been found responsible for the diverse mechanisms of dyserythropoiesis [11]. Since our patient had normal karyotype when he was diagnosed, high-resolution techniques such as chromosomal microarray might reveal small mutations. However, when diagnosing MDS, morphologic criteria can be more important and informative [12]. Della Porta et al. [13] performed a systematic review to identify a solid criteria to define dysplasia for diagnosing MDS. For erythroid dysplasia, significant correlations were found with the number of dysplastic erythroblasts. On the other hand, there were no significant correlations with the cytogenetic risks stratified according to the MDS cytogenetic scoring system or the number of somatic mutations.

In this study, we were unable to confirm whether the cup-shaped RBC is a dyserythropoietic feature. Study samples from MDS patients as well as patients with all kinds of anemia must be examined for poikilocytes in 3-D images. The ODT setup microscope can be a more sensitive tool for observing poikilocytes compared to the light microscope. Less than 5% of stomatocytes were observed in the patient’s peripheral blood smear whereas more than 30% were cup-shaped RBCs in 3-D images. However, RBC transfusion cannot be ruled out as a cause of the shape change observed in the cells. More RBCs from MDS patients and normal RBCs from
the reference group are thus needed for further studies. The rapid and precise imaging ability of ODT enabled direct measurement of the 3-D morphology of these cup-shaped RBCs from the MDS patient [14]. Furthermore, the label-free feature of ODT can be potentially useful for the rapid diagnosis of MDS and related diseases.

In conclusion, the cup-shaped RBCs may be considered as a dysplastic feature of MDS in the peripheral blood, which was not observed by light microscopy in this study. Further studies with a large population are absolutely needed to support this hypothesis. However, if confirmed, cup-like RBCs can be helpful to identify dysplastic features of MDS in peripheral blood. Nonetheless, it is obvious that 3-D images of RBCs are definitely helpful and more sensitive for detecting poikilocytes or dysplastic features compared to 2-D images.

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

YongKeun Park has financial interests in Tomocube Inc., a company that commercializes optical diffraction tomography instruments.

REFERENCES