의학석사 학위논문

Characterization of red blood cells in myelodysplastic syndrome using optical diffraction tomography setup microscope

3 차원 회절광 현미경을 이용한 골수이형성증후군 환자의 적혈구 분석

울산대학교대학원

의 학 과

구 세 은

의학석사 학위논문

Characterization of red blood cells in myelodysplastic syndrome using optical diffraction tomography setup microscope

3 차원 회절광 현미경을 이용한 골수이형성증후군 환자의 적혈구 분석

울산대학교대학원

의 학 과

구 세 은

Characterization of red blood cells in myelodysplastic syndrome using optical diffraction tomography setup microscope

지 도 교 수　　장 성 수

이 논문을 의학석사 학위 논문으로 제출함

2018 년 08 월

울 산 대 학 교 대 학 원

의　학　과

구 세 은

구세은의 의학석사학위 논문을 인준함

심사위원　박 찬 정　(인)

심사위원　장 성 수　(인)

심사위원　조 영 욱　(인)

울 산 대 학 교 대 학 원

2018 년　08 월

ABSTRACT

Myelodysplastic syndrome (MDS) is a clonal hematopoietic neoplasm which its etiology and pathogenesis are unclear. Moreover, vague pathogenesis, various phenotypes, and genetic mutations make it hard to diagnose. Currently, there is no definite clue to rule out MDS from other hematologic diseases from the patient's peripheral blood. Bone marrow examination must be done to diagnose MDS. Still, there are limitations to distinguish from other disease and there are no definitive diagnostic criteria that can clearly predict the prognosis of MDS.

Optical diffraction tomography (ODT) uses interferometric microscopy technique which can measure 3-D refractive index (RI) distribution of any samples directly without labeling procedure. This can also measure important parameters of individual RBC with the programmed algorithm such as corpuscular hemoglobin content (CH), corpuscular hemoglobin concentration (CHC) and corpuscular volume (CV). Also, unique individual RBC parameters can be measured which is not possible to obtain from commercially automated hematology analyzer, such as diameter, membrane fluctuation, surface area (SA) and surface index (SI) of an RBC. SI represents the sphericity of a cell defined as a normalized volume-to-surface area ratio. It ranges from 0 to 1, flat disks to perfect spheres, respectively. The fluctuation is a very exclusive parameter to get it under the microscope. It is obtained by continuously and fast recording 2-D RI phase images using a high-speed camera. The fluctuation of RBC membranes is acutely affected by diverse pathophysiological conditions. Therefore, we predicted the fluctuations from the MDS RBCs will be decreased because there should be unnoticeable damages done to RBC membranes caused by dyserythropoiesis.

With help of this microscope, we tried to find characteristics or different aspects of MDS RBCs in peripheral blood samples which can be helpful to diagnose MDS or to elucidate pathogenesis little more than the present.

We collected two groups of blood samples from EDTA anticoagulant tube which were left-overs from complete blood count tests done by Asan laboratory and

diagnostic medicine department (Seoul, Korea). One group was from patients diagnosed with MDS and the other group was from healthy individuals who visited our hospital for a regular health check-up. We also collected samples with patients having cytopenia on their complete blood count (CBC) results including MDS patients to find out where there are any different morphologic features that we cannot distinguish on the routine blood smear examination using Wright staining method and light microscope. RI tomograms were measured using ODT setup microscope, HT-1S, and HT-1H (Tomocube Inc., Daejeon, South Korea). For the visualization of the measured 3-D RI tomograms and obtaining RBC parameters, commercial software (Tomostudio, Tomocube, Inc., Daejeon, South Korea) was used.

Correlation maps were used between RBC parameters. And two sample Welch's t-test was used for comparison of RBC parameters between MDS patients and healthy individuals.

Total 220 MDS RBCs, 240 normal RBC cells were analyzed to obtain 6 RBC parameters. Each parameter was separately visualized (Fig. 1) and analyzed (Table 1). There were statistical differences in CH, CHC, CV and SI ($P<0.05$). The means in CH, CV, CHC, and SI were higher in MDS RBCs. The SA and membrane fluctuation were not statistically different.

The relationship between two parameters of the six in individual RBC was visualized. CH-CV, CH-CHC, CH-SA, CV-SA were directly proportional and SA-SI was inversely proportional to each other. The result was same when analyzed normal RBCs and MDS RBCs separately. More RBCs must be analyzed to get trustworthy results.

There was a distinct morphological difference in RBCs obtained from one of our MDS patients who had dyserythropoietic features in the bone marrow. Unlike other anemic features of RBC that we were aware of such as target cells or increased central pallor area, it was a new morphologic peculiarity which shaped like a cup. This could be the result of decreased membrane elasticity of RBC membrane.

Individual RBC parameters could be measured easily and fast using ODT setup microscope. The correlations between these parameters were rational. However further study with more test samples is needed to obtain reliable measurements.

Being able to observe 3-D images of RBCs was a great strength of the ODT microscope. It wouldn't be possible to discover our new morphologic characteristics during our routine peripheral blood smear examinations which can be viewed only the upper part of the RBCs.

Therefore, ODT setup microscope is a powerful and fast tool to investigate the morphology of cells including erythrocytes. This microscope could aid commercially automated hematology analyzer when the CBC results and peripheral blood smear slides are doubtful and need to be reviewed by hematology experts. Furthermore, it can elucidate the pathogenesis of MDS by obtaining 3-D images from live blood cells.

KEY WORDS: myelodysplastic syndrome, RBC morphology, RBC parameter, optical diffraction tomography setup microscope

Content

List of tables

List of figures

INTRODUCTIONS

Myelodysplastic syndrome (MDS) is a clonal hematopoietic neoplasm which its etiology and pathogenesis are unclear. Moreover, vague pathogenesis, various phenotypes, and genetic mutations make it hard to diagnose[1)]. Currently, there is no definite clue to rule out MDS from other hematologic diseases from the patient's peripheral blood. Bone marrow examination must be done to diagnose MDS. Still, there are limitations to distinguish from other disease and there are no definitive diagnostic criteria that can clearly predict the prognosis of MDS.

Optical diffraction tomography (ODT) setup microscope uses interferometric microscopy technique which can measure 3-D refractive index (RI) distribution of any samples directly without labeling procedure[2)]. With RI tomogram, it can give lots of information about each cell in a sample including the 3-D view of its cells[3)], morphological, biochemical and mechanical parameters. In order to obtain 3-D cell image, scanning electron microscope (SEM) was usually used[10)] which takes time and money. Reconstructing RI map can make 3-D live-cell image fast and easily. Moreover, this microscope can measure important parameters of individual RBC with the programmed algorithm such as corpuscular hemoglobin content (CH), corpuscular hemoglobin concentration (CHC) and corpuscular volume (CV). Also, unique individual RBC parameters can be measured which is not possible to obtain from commercially automated hematology analyzer, such as diameter, membrane fluctuation, surface area (SA) and surface index (SI) of an RBC[2)]. SI represents the sphericity of a cell defined as a normalized volume-to-surface area ratio. It ranges from 0 to 1, flat disks to perfect spheres, respectively. The membrane fluctuation is a very exclusive parameter to get it under the microscope. It is obtained by continuously and fast recording 2-D RI phase images using a high-speed camera. The fluctuation of RBC membranes is acutely affected by diverse pathophysiological conditions such as diabetes mellitus. The membrane fluctuation was either decreased or increased compared it with the normal RBCs'[4,5)]. The deformity of RBC is

determined by various properties such as membrane composition, cytoplasm viscosity, spectrin network, shape, and size[4].

Hence, looking into RBC parameters and morphology is important to understand its pathophysiology. We predicted that many parameters from the MDS RBCs will be different from the normal RBCs' because there should be unnoticeable damages or structural changes from the dyserythropoiesis. With help of this microscope, we tried to find characteristics or different aspects of MDS RBCs in peripheral blood samples which cannot be noticed with the conventional method. We looked into interrelationships between the parameters, compared RBC measurements with normal group's, examined 3-D reconstructed morphology of RBCs.

MATERIALS AND METHODS

1. Sample preparations and Ethics statements

The studies were conducted according to the principles of the Declaration of Helsinki and were approved by the responsible ethics committee of Asan medical center (IRB project number: 2018-0071, 2018-0072).

We collected two groups of blood samples from EDTA anticoagulant tube which were left-overs from complete blood count tests done by Asan laboratory and diagnostic medicine department (Seoul, Korea). One group was from patients diagnosed with MDS and the other group was from healthy individuals who visited our hospital for a regular health check-up. We also collected samples with patients having cytopenia on their complete blood count (CBC) results including MDS patients to find out where there are any different morphologic features that we cannot distinguish on the routine blood smear examination using Wright staining method and light microscope. Collected blood (5μL) was diluted with Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium and magnesium (1mL) (Thermo Fisher, MA, USA). The diluted blood was loaded on a coverslip of 25 × 50 mm (C025501, MATSUNAMI GLASS Ind., LTD., JAPAN) then is topped by another same coverslip. RBCs which were sedimented nicely to the coverslip were randomly selected. For the RBC parameters, total 220 MDS RBCs, 240 normal RBC cells were collected from 7 and 8 individuals, respectively. Samples from four cytopenia patients and two healthy people were used to reconstruct 3-D images of RBCs. The underlying diseases in patients with cytopenia were MDS with excess blasts-2, hepatitis B virus related liver cirrhosis, diabetes mellitus nephropathy and liver abscess and end-stage renal disease, respectively.

2. Optical diffraction tomography setup microscope and image processing

RI tomograms were measured using commercialized ODT setup microscope, HT-1S, and HT-1H (Tomocube Inc., Daejeon, South Korea). For the visualization of the measured 3-D RI tomograms and obtaining RBC parameters, commercial software (Tomostudio, Tomocube, Inc., Daejeon, South Korea) was used.

3. Retrieval of RBC parameters

From 3-D RI tomogram and 2-D dynamic phase maps, 6 parameters can be obtained either directly or indirectly. Hemoglobin concentration in RBC is linearly proportional to the RI difference between the cell and the medium: $[Hb] = h\Delta ni/\alpha$. α is a refraction increment and has a value of 0.2 mL/g for hemoglobin[6]. The Hb contents were calculated by multiplying hemoglobin concentration by RBC volume. The volume and surface area of an RBC is measured directly from the 3-D RI tomogram. The surface index represents the sphericity of a cell defined as a normalized volume-to-surface area ratio: $[SI = \pi^{1/3}(6V)^{2/3}/S]$. The membrane fluctuation is acquired by averaging values from the formula: $[<h(h(x, y, t) - <h(x, y, t)>_{temporal})^2>_{temporal}]^{1/2}$, using 2-D dynamic phase images[2,4].

4. Statistical Analysis

Correlation maps were used to analyze relationships between RBC parameters. Two sample Welch's t-test was used for comparison of RBC parameters between MDS patients and healthy individuals. Differences between two groups were considered statistically significant at $P<0.05$. We performed these analyses using R[7] via RStudio[8]. To read data from the Excel file, the package gdata[9] is used.

RESULTS

1. Comparison of RBC parameters between normal group and MDS group

Total 220 MDS RBCs, 240 normal RBC cells were analyzed to obtain 6 RBC parameters. Each parameter was separately visualized (Fig. 1) and analyzed (Table 1). There were statistical differences in CH, CHC, CV and SI ($P<0.05$). The means in CH, CV, CHC, and SI were higher in MDS RBCs. The SA and membrane fluctuation were not statistically different.

The relationship between two parameters of the six in individual RBC was visualized (Fig. 2). CH-CV, CH-CHC, CH-SA, CV-SA were directly proportional and SA-SI was inversely proportional to each other. The result was same when analyzed normal RBCs and MDS RBCs separately.

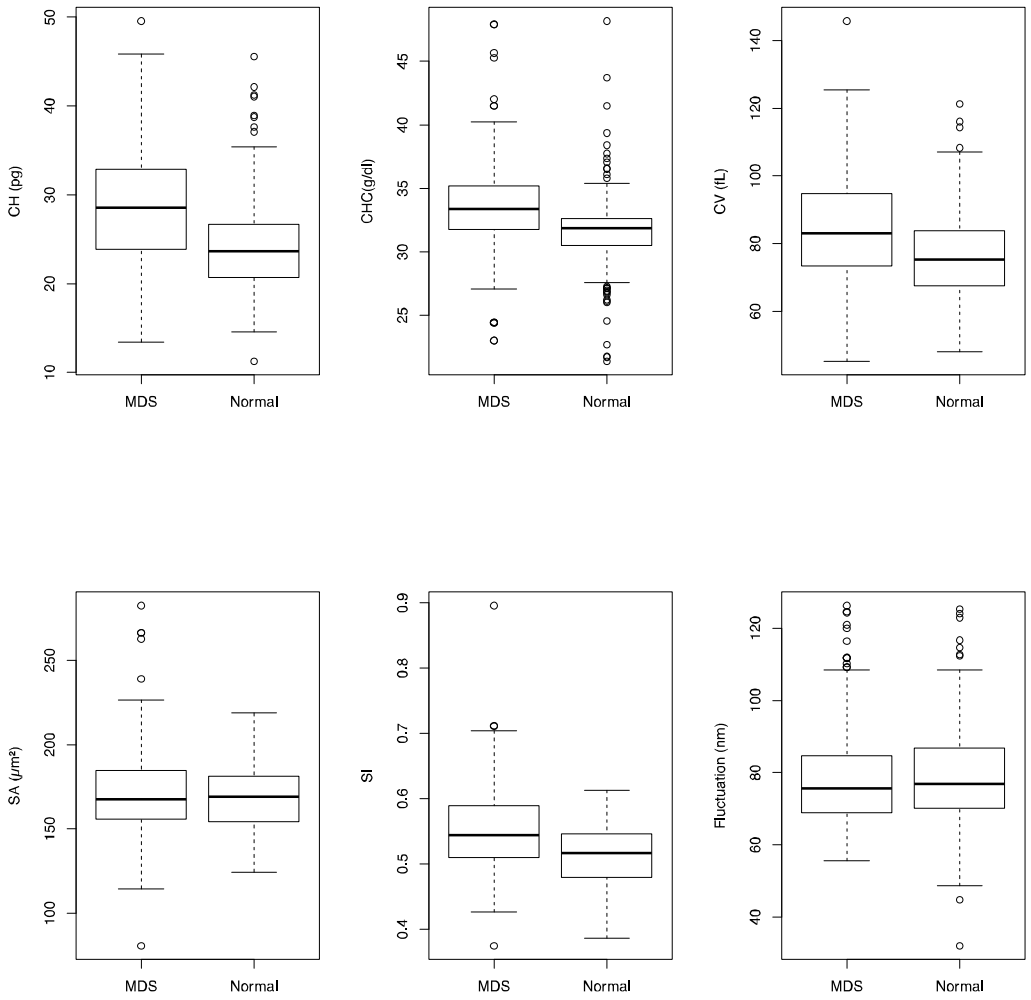

Fig. 1. Box plot diagrams of RBC parameters obtained from MDS patients and normal groups

Table 1. A summary of comparisons of RBC parameters obtained from MDS patients and normal groups using Welch Two Sample t-test

RBC parameters	Means in Normal RBCs	Means in MDS RBCs	Mean Difference	95% CI Lower	95% CI Upper	t	df	p-value
CH (pg)	24.13	28.70	-4.57	-5.68	-3.46	-8.12	453.30	<0.001*
CV (fL)	75.74	84.74	-9.01	-11.67	-6.34	-6.64	449.85	<0.001*
CHC(g/dl)	31.70	33.74	-2.04	-2.69	-1.40	-6.27	457.86	<0.001*
SA (µm²)	169.07	170.45	-1.38	-5.54	2.77	-0.65	427.12	0.513
SI	0.51	0.55	-0.04	-0.05	-0.03	-7.51	432.92	<0.001*
Fluctuation (nm)	78.65	80.09	-1.45	-4.44	1.54	-0.95	439.46	0.342

*p<0.05

Abbreviations: CH, corpuscular hemoglobin content; CHC, corpuscular hemoglobin concentration; CV, corpuscular volume; SA, surface area; SI, surface index.

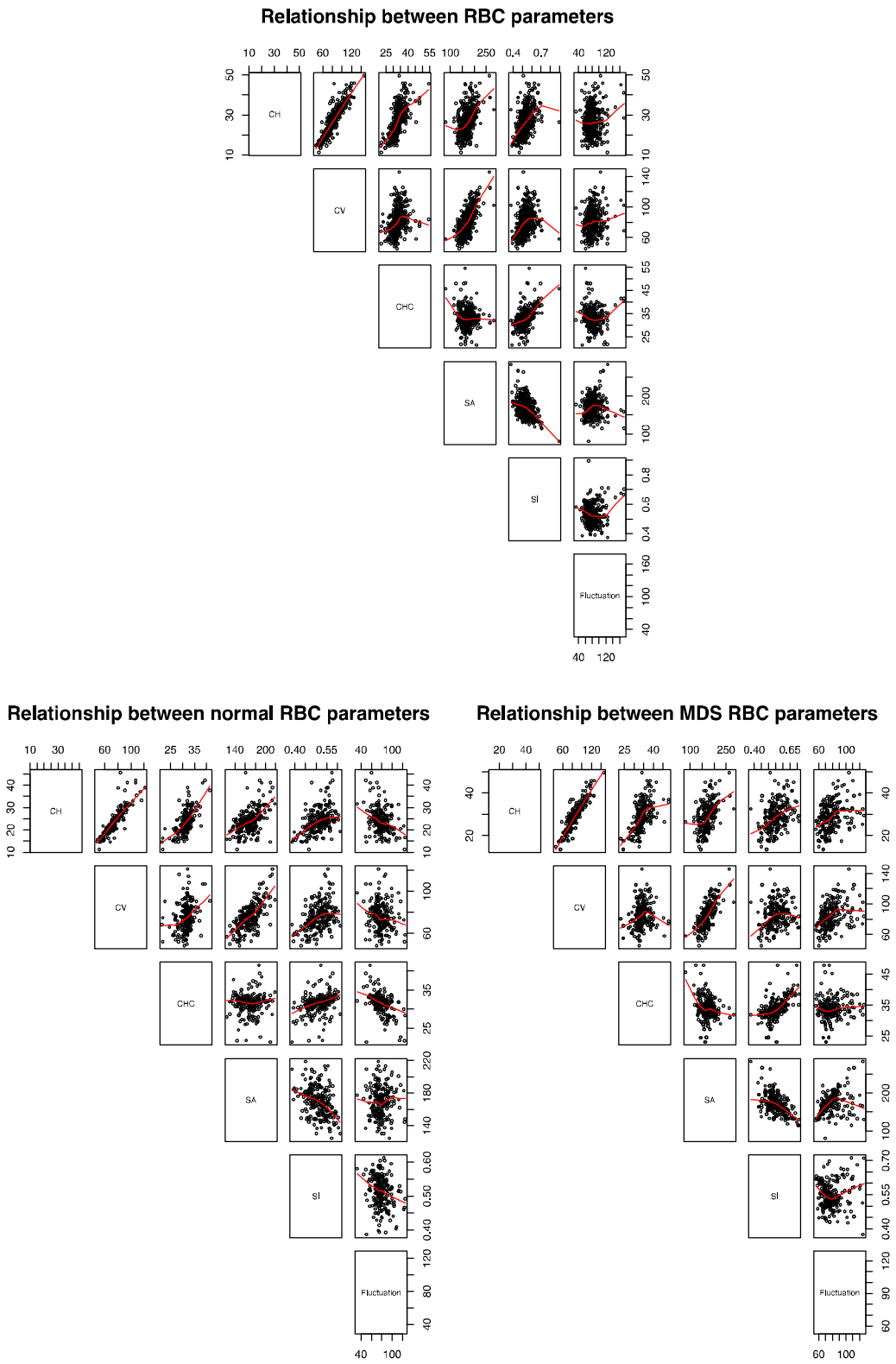

Fig. 2. Correlation maps between RBC parameters obtained from all RBCs including MDS patients and normal groups, only MDS groups and only normal groups.

2. Reconstructed 3-D images of RBC

The conventional method, making and staining peripheral blood smear slides and examining via light microscope were performed for a comparison (Fig. 3). There was no significant morphological difference between anemic RBCs except the one from the patient with liver cirrhosis. All four slides show RBCs with increased central pallor area. The samples from the patients with liver disease (Fig. 3 B and Fig.3) had target cells. Other than that, distinguishing MDS RBCs from other anemic RBCs was not possible. On the other hand, obtaining 3-D RBCs using an ODT setup microscope showed significant differences. With the front view, the shape of RBCs looked all same. However, the side view showed remarkable dissimilarity (Fig. 4). There was a distinct morphological difference in RBCs obtained from the MDS patient (Fig. 4 A). He was diagnosed with MDS with excess blasts-2 10 months ago. Recent bone marrow exam showed dyserythropoietic feature. Unlike other anemic features of RBC that we were aware of such as target cells or increased central pallor area, it was a new morphologic peculiarity which shaped like a cup.

For further evaluation, 40 and 61 3-D RBC images from this patient are reviewed. The samples were collected when he visited our hospital for his outpatient follow-up on May 21, 2018, and May 29, 2018, respectively (Fig 5 A and B). 48 and 59 RBCs from healthy individuals are also analyzed on the same day for the healthy reference (Fig 5 C and D). As a result, 37.5% and 32.8% of RBCs from the MDS patient had cup-shaped RBCs where none are found in the normal group's (Table 2).

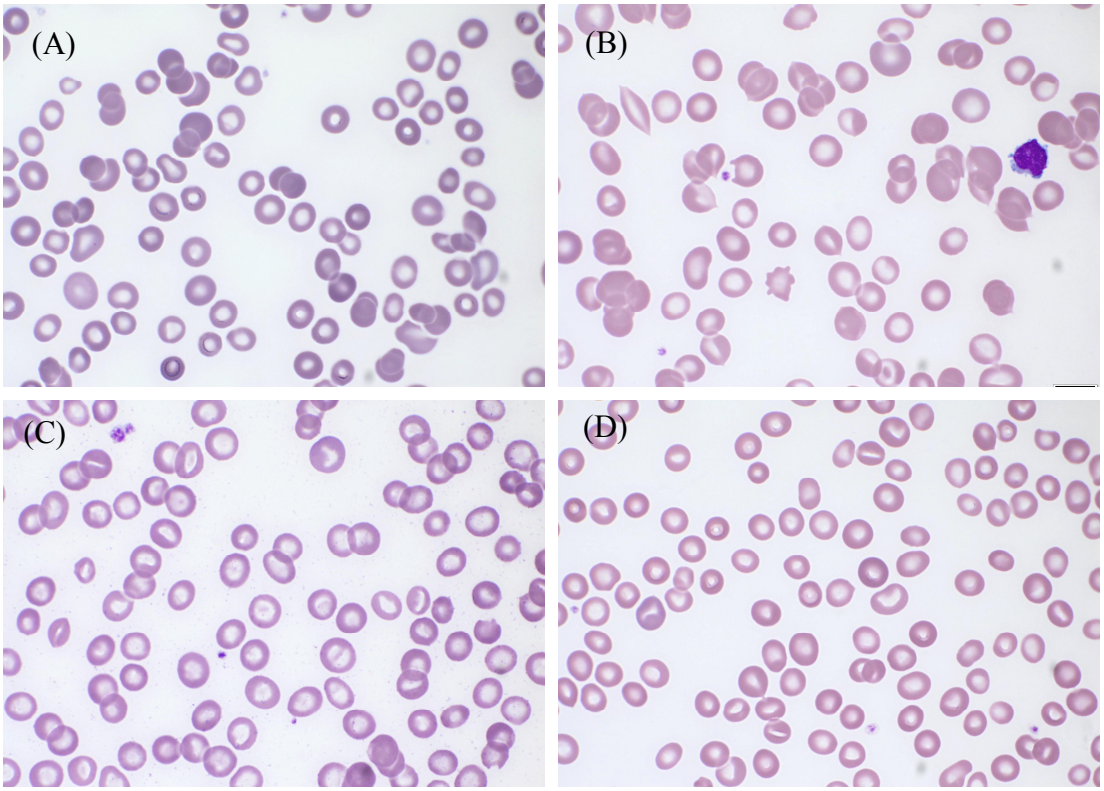

Fig. 3. Peripheral blood findings of patients with bicytopenia using a light microscope (Wright stain, ×1,000). The underlying disease is different from one another: (A) Myelodysplastic syndrome with excess blasts-2, (B) hepatitis B virus related liver cirrhosis, (C) Diabetes mellitus nephropathy and liver abscess (D) end stage renal disease.

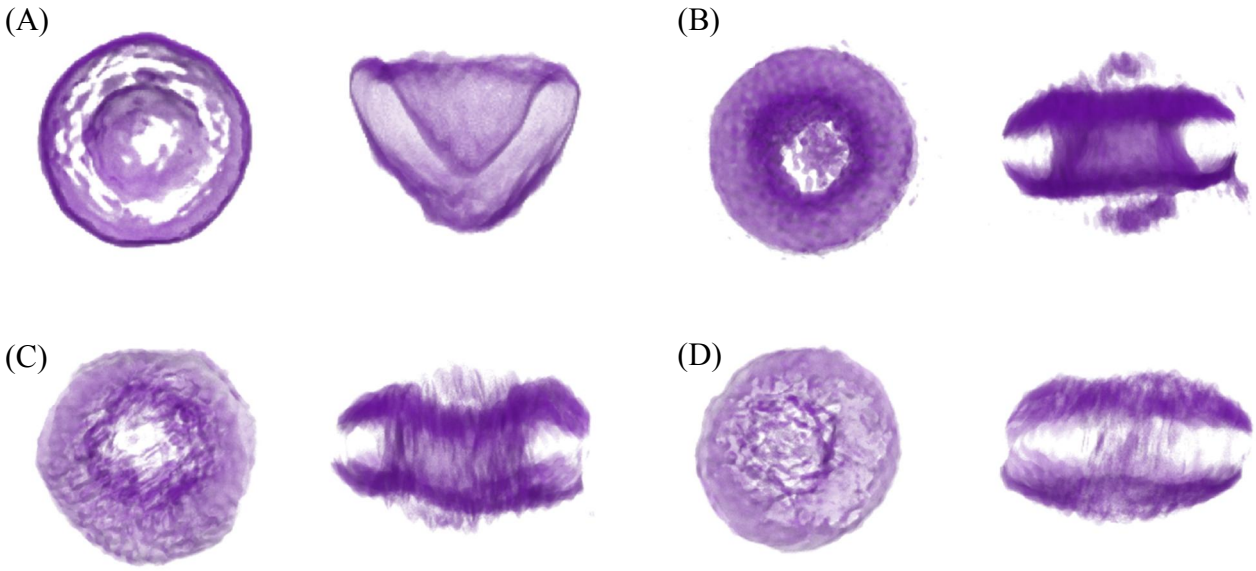

Fig. 4. Front and side views of reconstructed 3-D RBC images from patients with bicytopenia using an ODT setup microscope, HT-1S (Tomocube Inc., South Korea) and a commercial software (Tomostudio, Tomocube, Inc., South Korea). The underlying disease is different from one another: (A) Myelodysplastic syndrome with excess blasts-2, (B) hepatitis B virus related liver cirrhosis, (C) Diabetes mellitus nephropathy and liver abscess (D) end stage renal disease. The cell sizes are not relative to each other

(A)

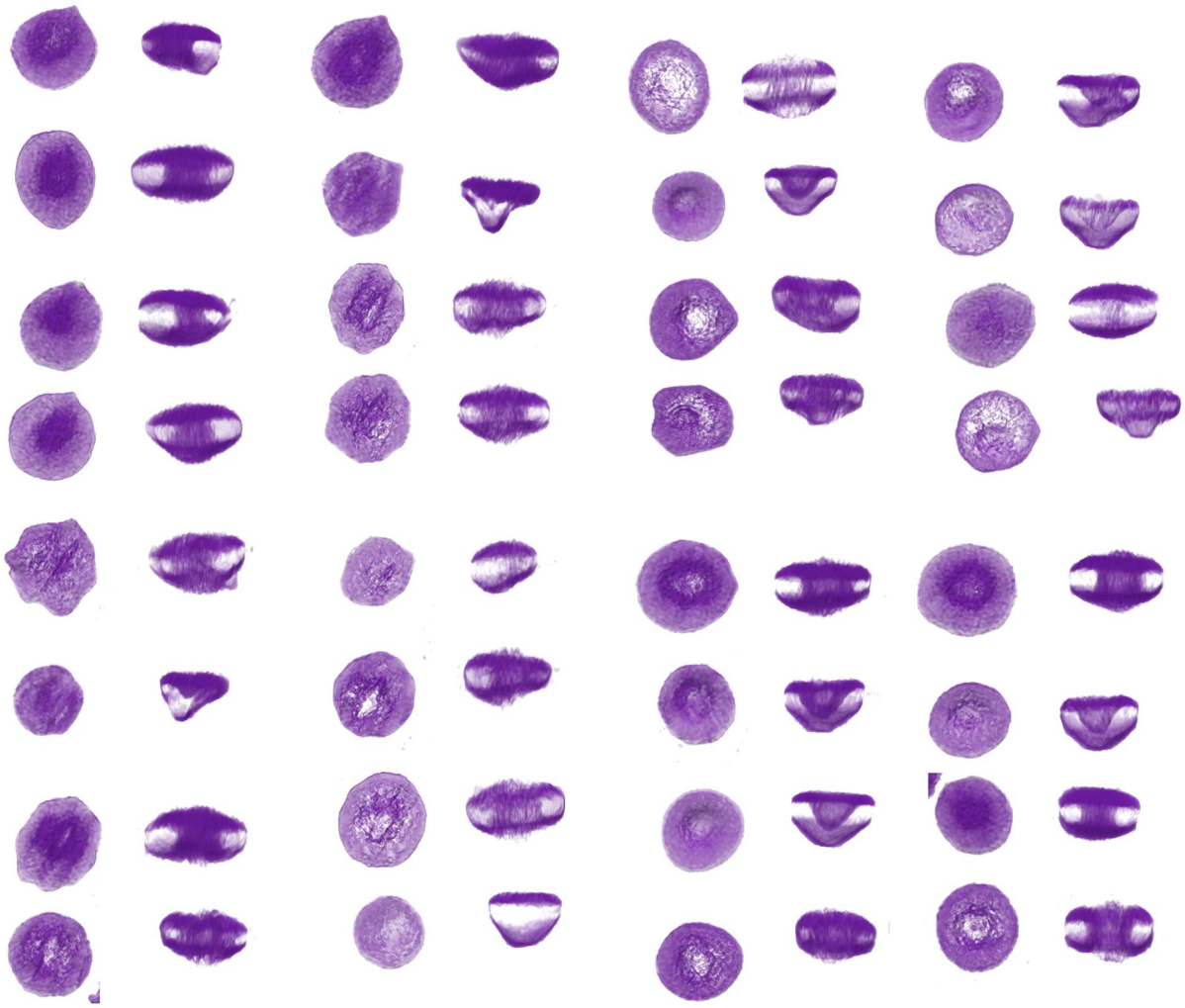

Fig. 5. Front and side views of reconstructed 3-D RBC images from the patient diagnosed with MDS with excess blasts-2 during his outpatient follow-up on May 21, 2018 (A) and May 29, 2018 (B). Healthy references were also reviewed on May 21, 2018 (C) and May 29, 2018 (D). The cell sizes are not strictly relative to each other.

Fig. 5. *(Continued)*

(B)

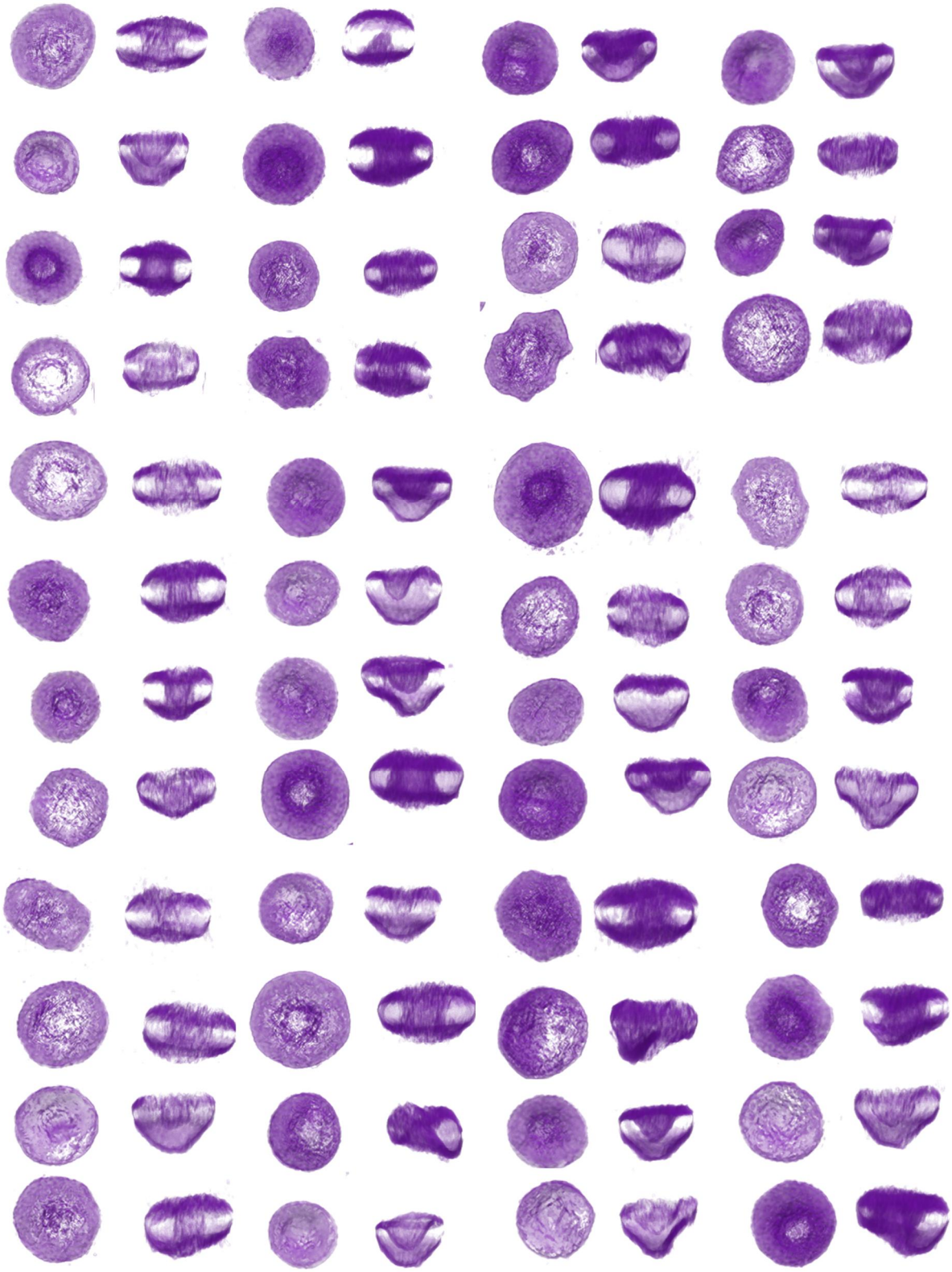

Fig. 5. *(Continued)*

(B) *(Continued)*

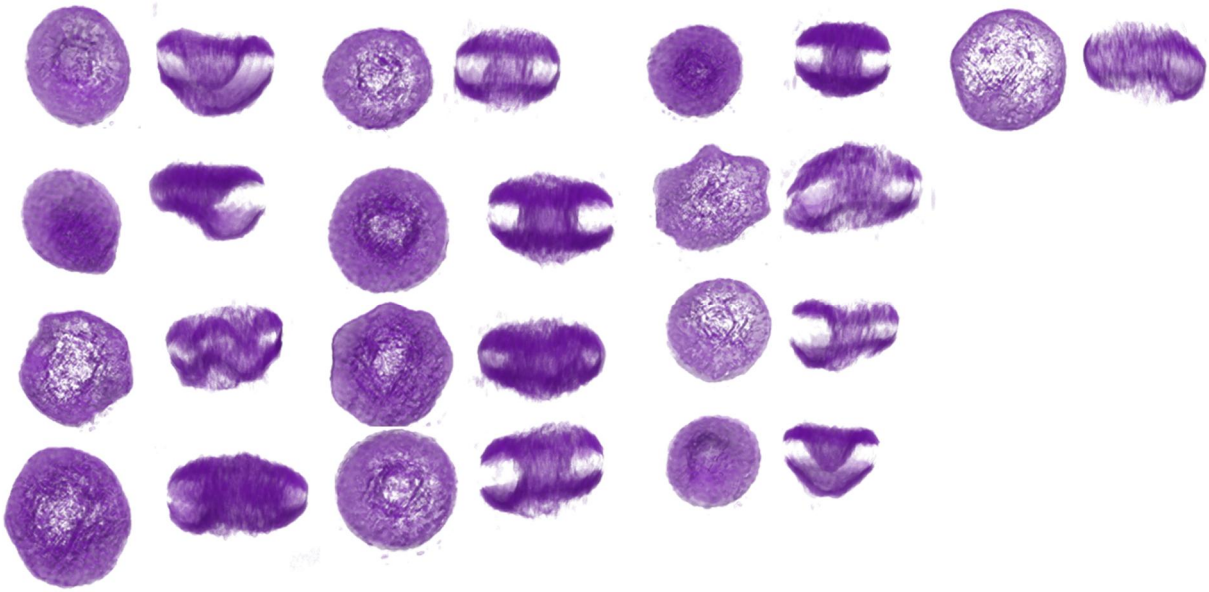

Fig. 5. *(Continued)*

(D)

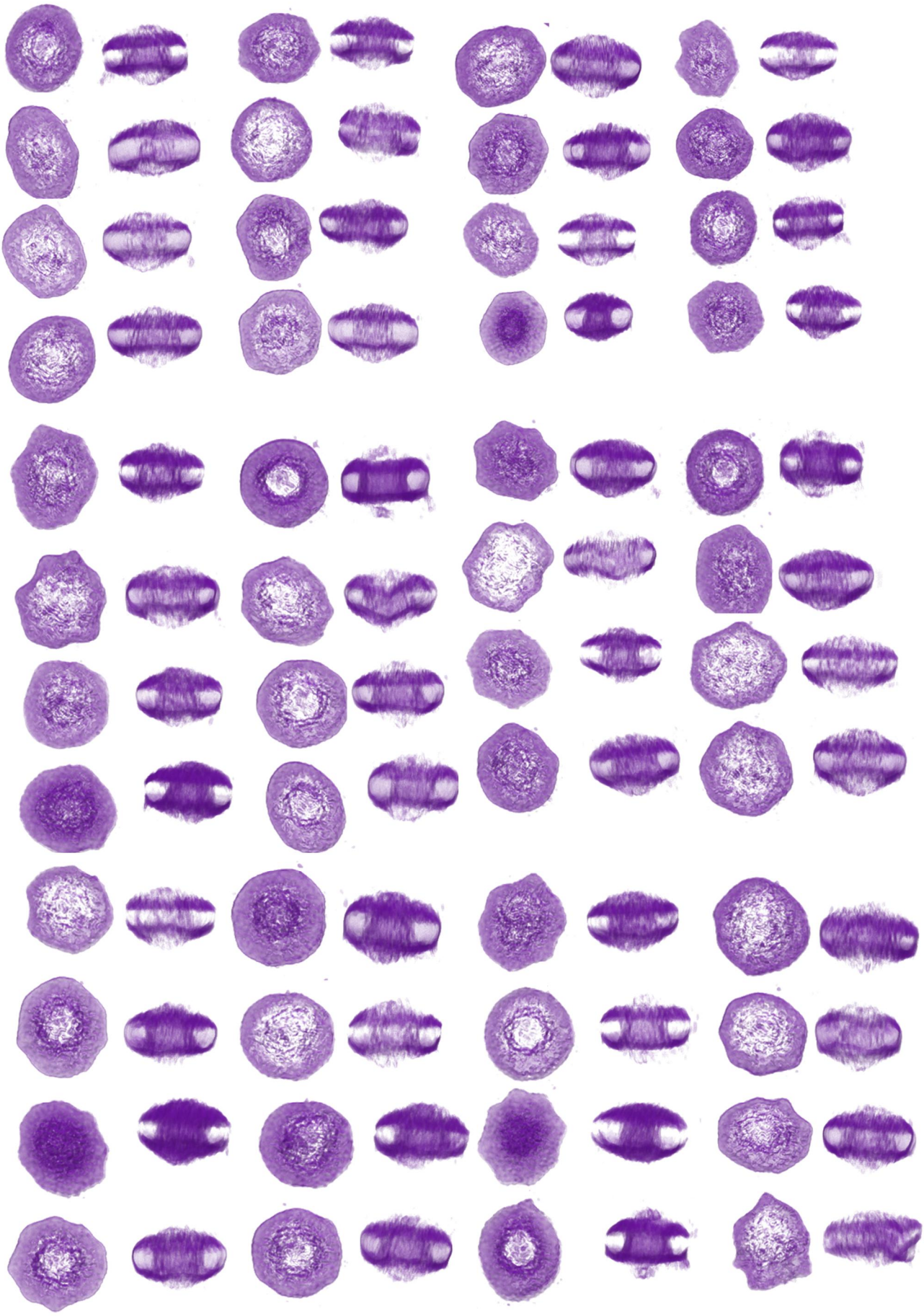

Fig. 5. *(Continued)*

(D) *(Continued)*

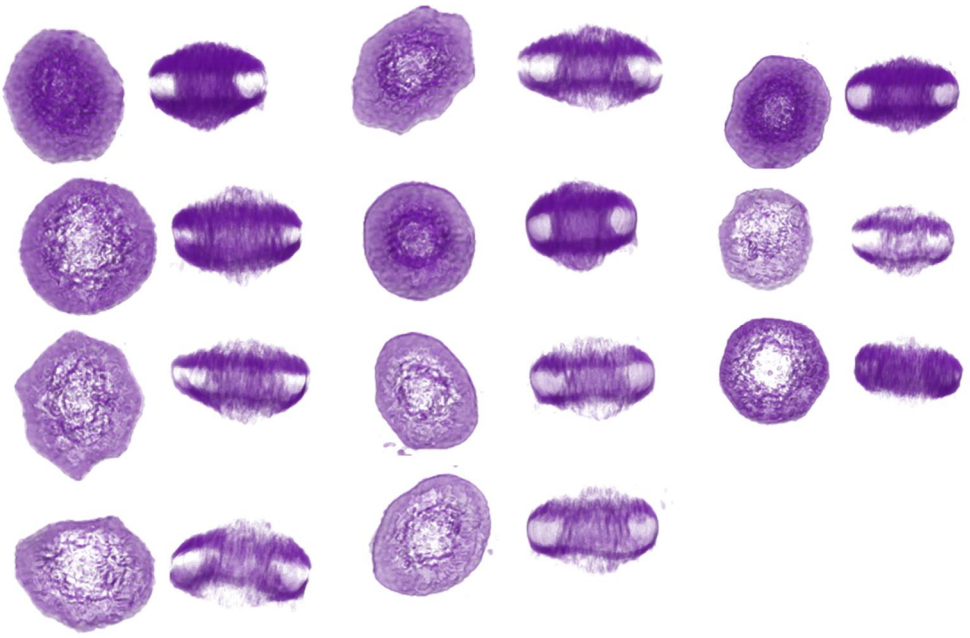

Table 2. RBC counts (with percentage shown in parentheses) by its morphology in each sample. The samples are from the patient diagnosed with MDS with excess blasts-2 during his outpatient follow-up on May 21, 2018 (A) and May 29, 2018 (B), healthy references on May 21, 2018 (C) and May 29, 2018 (D). (with percentage shown in parentheses)

*Cup-like shape RBCs are not included

RBC Samples	Cup-shape	Abnormal shape*	Normal shape	Total RBCs
(A)	15 (37.5)	2 (5.0)	23 (57.5)	40 (100)
(B)	20 (32.8)	5 (8.2)	36 (59.0)	61 (100)
(C)	0 (0)	2 (4.2)	46 (95.8)	48 (100)
(D)	0 (0)	2 (3.4)	57 (96.6)	59 (100)

DISCUSSIONS

Being able to directly measure individual RBC parameters and view 3-D RBC morphologies by just loading a drop of diluted blood on a coverslip was sensational. The correlation between parameters was rational. There were no interesting findings. There were no differences between normal RBCs and MDS RBCs when it comes to relationships between parameters. However, breaking down MDS RBCs into more subgroups based on subtypes in WHO Classification of MDS or cytogenetics could give a valuable discovery.

Getting individual RBC parameters can be helpful. Conventional automated hematology analyzer gives only the mean value of RBCs such as MCV. This can mask the abnormal results when the normal cells or reticulocytes are present. The relationship between parameters were rational and nothing was different with MDS RBCs. MDS RBCs having high CH and CV were expected results. Having macrocytosis without increased blasts in MDS patient could be a good prognostic factor[14,15]. Using the advantage that MCV or CH can be measured on each RBCs, the relationships between proportion of macrocytes and long-term survival could be a meaningful study. The CHC were lower in MDS RBCs which did not make sense. More studies with more RBCs and adjusting the parameters with conventional CBC results in needed. High in SI means MDS RBCs tend to be more spherical than the normal RBCs. Meaning when the RBC volumes are same, MDS RBCs have less surface area than the normal RBCs, ineffective in oxygen delivery. The cell shape and structural change in RBCs have a strong effect on the membrane fluctuations. There should be a difference in MDS RBCs. However, measuring this parameter is challenging. It is very sensitive to the surroundings, even speaking while processing would give the wrong result. RBCs which is not intact with the cover glass will float around. These RBCs are not measurable. Therefore, careful processing and choosing perfectly fixed RBCs for analyzing is required.

Easily obtaining 3-D images of RBCs was a great strength of the ODT microscope. It wouldn't be possible to discover unique morphologic characteristics during our routine peripheral blood smear examinations which can be viewed only the upper part of the RBCs. Only pancytopenia with increased MCV and red blood cell distribution width (RDW) was observed in the patient's Wright-stained peripheral blood slide. The cup-shaped RBCs can be one of dyserythropoietic feature that can be seen in peripheral blood. This patient showed dyserythropoiesis in the bone marrow such as binucleation. The RBC membrane consists of lipid bilayer and spectrin network. The spectrin network makes a resistance to the bending force and the shear strain and maintains the shape of the RBC. It is tightly attached to the lipid bilayer in normal RBCs under the normal conditions. This tight and elastic structure can become dissociated in pathologic conditions[11]. The cup shape can be the resulting of losing elasticity after denucleation process in the bone marrow. Also, genetic alteration plays major role in making dysplastic RBCs. 5q deletion, for example, affects ribosomal protein gene RPS14, leading to hinder erythrocyte maturation. Many mutations were being found responsible for the the diverse mechanisms of dyserythropoiesis[12]. However, the patient had normal karyotype when he was diagnosed. High resolution technique such as chromosomal microarray should be performed for detecting small mutations. At this moment, the cup-shape RBCs were only found in one MDS patient. Patients who were diagnosed with MDS and have dyerthropoietic features in the bone marrow should be checked with the ODT setup microscope to obtain 3-D RBC morphology.

Overall, more samples are needed to obtain reliable measurements. And analyzing RBCs more specifically by their subtypes should be needed in the farther study.

CONCLUSION

ODT setup microscope is a powerful and fast tool to investigate the morphology of cells including erythrocytes real time. This microscope could aid commercially automated hematology analyzer when the CBC results and peripheral blood smear slides are doubtful and need to be reviewed by hematology experts. Furthermore, it can elucidate the pathogenesis of MDS by obtaining 3-D images and parameters from live individual blood cells.

The cup-shaped RBC has the potential to be the important and only morphological feature found in the peripheral blood for determining erythroid lineage dysplasia. At this moment, the cup-shaped RBCs were only found in one MDS patient. Patients who were diagnosed with MDS and have dyerthropoietic features in the bone marrow should be checked with the ODT setup microscope to obtain 3-D RBC morphology for the further study.

REFERENCES

1. Hasserjian RP, Head DR. Hematopathology 2nd Edition, Ch. 45 Myelodysplastic Syndromes. 2016.
2. Kim Y, Shim H, Kim K, Park H, Jang S, Park Y. Profiling individual human red blood cells using common-path diffraction optical tomography. Sci Rep [Internet]. 2014;4:6659. Available from: http://www.nature.com/articles/srep06659
3. Kim K, Kim KS, Park H, Ye JC, Park Y. Real-time visualization of 3-D dynamic microscopic objects using optical diffraction tomography. Opt Express [Internet]. 2013;21(26):32269. Available from: https://www.osapublishing.org/abstract.cfm?URI=oe-21-26-32269
4. Lee SY, Park HJ, Best-Popescu C, Jang S, Park YK. The effects of ethanol on the morphological and biochemical properties of individual human red blood cells. PLoS One. 2015;10(12):1–14.
5. Lee S, Park H, Kim K, Sohn Y, Jang S, Park Y. Refractive index tomograms and dynamic membrane fluctuations of red blood cells from patients with diabetes mellitus. bioRxiv [Internet]. 2016;(March):1–11. Available from: http://biorxiv.org/content/early/2016/11/14/087460.abstract
6. Park Y, Yamauchi T, Choi W, Dasari R, Feld MS. Spectroscopic phase microscopy for quantifying hemoglobin concentrations in intact red blood cells. Opt Lett. 2009; 34(23):3668–70. Epub 2009/12/03. doi: 10.1364/OL.34.003668 PMID: 19953156; PubMed Central PMCID: PMC2848941.
7. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
8. Team R. RStudio: Integrated Development for R. Boston: RStudio Inc.; 2015. Version 1.1.453
9. Gregory R. Warnes, Ben Bolker, Gregor Gorjanc, Gabor Grothendieck, Ales Korosec, Thomas Lumley, Don MacQueen, Arni Magnusson, Jim Rogers and others

(2017). gdata: Various R Programming Tools for Data Manipulation. R package version 2.18.0. https://CRAN.R-project.org/package=gdata

10. Salsbury AJ, Clarke JA. New method for detecting changes in the surface appearance of human red blood cells. 1967;603–11.

11 Peng Z, Li X, Pivkin I V, Dao M, Karniadakis GE, Suresh S. Lipid bilayer and cytoskeletal interactions in a red blood cell. Proc Natl Acad Sci U S A. 2013;110(33).

12. Lefèvre C, Bondu S, Le Goff S, Kosmider O, Fontenay M. Dyserythropoiesis of myelodysplastic syndromes. Curr Opin Hematol. 2017;24(3):191–7.

13. Della Porta MG, Travaglino E, Boveri E, Ponzoni M, Malcovati L, Papaemmanuil E, 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.

14. Matsuda, A., Mitsumi, M., Yoshida, K., Yagasaki, F., Ito, Y., Kawai, N. & Bessho, M. (2003) Is macrocytosis a favourable prognostic factor in myelodysplastic syndrome patients without increased blasts? British Journal of Haematology, 121, 815–816.

15. Tennant, G. B., Cavill, I. and Burnett, A. K. (2004), Long-term survival of myelodysplastic patients with macrocytosis. British Journal of Haematology, 124: 840-841. doi:10.1111/j.1365-2141.2004.04860.x

KOREAN ABSTRACT

골수이형성증후군 (MDS)는 클론성의 악성신생물로, 원인과 병태생리학적 기전이 명확하지 않다. 병태생리학적 기전의 불확실성, 표현형과 돌연변이의 다양성이 더더욱 정확한 진단을 힘들게 만든다. 현재로선, 골수검사를 통해서 진단을 하며 말초혈액에서는 진단을 할 수 있는 diagnostic criteria 가 없다.

Optical diffraction tomography (ODT) 현미경은 는 3-D refractive index (RI)를 측정할 수 있다. 샘플을 염색이나 전처리 없이 바로 측정을 하여 3 차원 이미지로 구현이 가능하다. 또한, corpuscular hemoglobin content (CH), corpuscular hemoglobin concentration (CHC), 그리고 corpuscular volume (CV) 와 같은 자동혈구분석장비에서는 평균값만 측정이 가능한 적혈구 지표들을 하나의 적혈구에서의 값을 구할 수 있다. 더불어, 적혈구의 직경, membrane fluctuation, surface area (SA) and surface index (SI)과 같은 자동혈구분석장비에선 측정 할 수 없는 값들을 구할 수 있다. 따라서 이 현미경을 이용하여, 골수이형성증후군 환자의 말초혈액 적혈구의 특징을 알아보려 하였다.

우리는 서울아산병원에서 검사하고 남은 EDTA 말초혈액 검체를 사용하였다. MDS 를 진단받은 환자의 검체를 실험군으로 이용하였고, 건강검진을 위해 내원한 환자의 검체를 대조군으로 사용하였다. 또한, MDS 환자들을 포함한 cytopenia 가 있는 여러 질환의 환자들의 검체를 가지고 3 차원 적혈구 이미지를 구현 하였다. 회절광 현미경은 HT-1S 와 HT-1H (Tomocube Inc., Daejeon, South Korea)를 사용하였다. 이미지 구현과 적혈구의 측정값을 구하기 위해서는 Tomostudio software (Tomocube, Inc., Daejeon, South Korea)를 이용하였다. Correlation maps 과 two sample Welch's t-test 를 사용하여 실험군과 대조군의 적혈구 parameter 값을 비교 하였다.

총 220 개의 MDS 적혈구와 240 개의 정상 적혈구를 분석하였다. CH, CHC, CV, 그리고 SI 값은 통계적으로 유의하게 차이가 있었다 ($P<0.05$). CH, CV, CHC, 그리고 SI 의 평균값은 MDS 적혈구에서 의미 있게 대조군의

값보다 높았다. SA 와 membrane fluctuation 값은 통계적으로 유의한 차이가 없었다.

비교군과 대조군의 적혈구에서 각각 두 parameter 의 관계를 보는 관계그래프에서는 CH-CV, CH-CHC, CH-SA, CV-SA 가 정비례하였고, SA-SI 의 관계는 반비례 하였다. 두 군간의 차이는 없었다.

Cytopenia 환자의 적혈구를 3 차원 이미지로 구현 하였는데, MDS 환자 중 한명의 검체에서 특이한 컵 모양의 적혈구가 관찰 되었다. 두번의 내원 당일의 적혈구를 측정 하였는데, 각각 37.5%와 32.8%의 적혈구가 컵모양을 보였다. 이는 MDS 환자의 적혈구 막의 탄력도가 떨어져서 생기는, 말초혈액에서 발견 할 수 있는 MDS 적혈구의 특이 소견일 수도 있을 것이다.

3 차원 회절광 현미경을 이용해 개개의 적혈구의 parameter 들을 측정하고 3 차원 이미지로 구현 하는 것은 빠르고 쉬웠다. 하지만 더 많은 환자의 검체를 갖고 분석이 필요하다. 이 현미경은 자동혈구분석장비를 보조하는 역할을 할 수도 있을 것이고, MDS 의 병태생리학적 기전을 밝히는 데에 도움을 줄 수 있을 것이다.