진단적 유리체절제술의 수율에 영향을 미치는 인자들: 유리체절제침 내경 및 절삭 속도

Factors Influencing the Yield of Diagnostic Vitrectomy: Ocutome Diameter and Cutting Rate

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Purpose: To evaluate the effects of ocutome diameter and cutting rate on the yield of diagnostic vitrectomy.

Methods: Blood samples were obtained from a 40-year-old healthy male and treated with ethylenediaminetetraacetic acid. Samples were aspirated under cutting rates of 0, 800, 1,200, 2,500, 5,000, and 8,000 cuts per minute (CPM) using a 25- or 27-gauge vitrectomy cutter. The white blood cell (WBC) break rate and hemolysis index were calculated to evaluate the quality of the specimen.

Results: A WBC count of 0-1.82% was unidentifiable in the collected blood using the 25-gauge ocutome, as was that of 0.87-8.00% under a 27-gauge ocutome. The ratio of unidentifiable WBCs increased with increasing cutting rate ($p = 0.030$). This ratio did not show any difference depending on diameter of the vitrectomy cutter ($p = 0.763$). The hemolysis index increased with the 25-gauge cutter compared to the 27-gauge. Both ocutome diameter and cutting rate affected the hemolysis index ($p < 0.001$, $p = 0.033$).

Conclusions: The results of the current study using blood samples showed that the small-gauge ocutome increased only the hemolysis index, while both the hemolysis index and the WBC break rate were higher at higher cutting rates. These results suggest that, when collecting a vitreous specimen by diagnostic vitrectomy, the ocutome diameter is not important. However, when collecting vitreous specimens at high CPM, attention is required because the hemolysis index and WBC break rate increase.

Keywords: Cutting rate; Diagnostic yield; Pars plana vitrectomy; Small gauge vitrectomy

Introduction

Diagnostic pars plana vitrectomy is useful for diagnosing intraocular malignancy and posterior segment inflammation [1-6]. The yield of diagnostic vitrectomy varies from 14.2%

to 64.3% depending on patient selection and clinical setting [7-13]. Wittenberg et al. [12] reviewed vitreous cytology obtained by diagnostic vitrectomy targeting 278 eyes from 225 people over 15 years and reported a diagnostic yield of 55.3%. In contrast, Oahalou et al. [13] reported a positive

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rate in 18 of 84 cases (21%).

Fujii et al. introduced commercialized 25-gauge vitrectomy in 2002 [14,15], and Oshima et al. [16] described 27-gauge vitrectomies in 2010. Small-gauge vitrectomy reduces the operation time compared to the 20-gauge standard vitrectomy, decreasing inflammation after surgery and improving patient comfort. It is widely used clinically [17-20]. In addition, according to recent development of vitreoretinal surgical technology, vitrectomy cutting rates have increased. Because a higher cutting rate results in a smaller bolus, the tractional force can be reduced during surgery. Particularly in diagnostic vitrectomy, when an undiluted vitreous sample is preferentially obtained, a higher cutting rate might enable safer surgery. In contrast, smaller fragments from higher cutting rates and smaller ocutome diameters can influence vitreous samples. Because the number of cells from vitreous biopsies is small, the diagnostic yield can decrease substantially if a small portion of cells is destroyed. To acquire undiluted vitreous samples during diagnostic vitrectomy, diagnostic yield can be increased by air infusion or perfluorocarbon perfusion in addition to adjusting the cutting rate or ocutome diameter [21,22].

In diagnostic vitrectomy, observation of WBC is often important. We hypothesized that the blood sample would be easily demonstrate the change in WBC according to the parameters controlled during vitrectomy. In this study, we investigated the effect of ocutome diameter and cutting rate on the yield of diagnostic vitrectomy using blood samples.

Materials and Methods

Blood samples were collected once for a total of 30 mL using an 18-gauge needle at room temperature from a 40-year-old healthy male and placed into 3-mL ethylene diaminetetraacetic acid-processed bottles. The experiment was conducted under sterile conditions at a standardized temperature and humidity in an operating room. An EVA[®] vitrectomy system (D.O.R.C, Kerkweg, Netherlands) with 25-gauge and 27-gauge ocutome was used. Blood samples were placed in an artificial chamber; 2 mL of the sample was aspirated at a rate of 0, 800, 1,200, 2,500, 5,000, and 8,000 cuts per minute (CPM) using a 10-mL syringe. Aspiration was conducted manually at a constant force to create the same environment as in vitreous sampling in the operating

room for 1 minute. The blood sample used in this study was obtained from one of the authors. Blood collection was performed voluntarily, and collection of small amounts of blood from young and healthy men was not expected to have harmful effects on the human body and was conducted without IRB approval. This study was conducted according to the ethical standards stated in the Declaration of Helsinki. All specimens were moved to the pathology laboratory as soon as they were collected and examined by one pathologist (H.S.M.). Two indexes were calculated to evaluate the quality of the specimen.

1) White blood cell (WBC) break rate: The WBC break rate was calculated by counting the number of unidentifiable WBCs within approximately 100 WBCs. Unidentifiable WBCs were those that were unrecognizable in shape due to a loss of cytoplasm or nuclear membrane rupture (Fig. 1). This was determined under a 400× magnification microscope after a blood smear and Wright Giemsa stain.

2) Hemolysis index: The formula used to calculate the hemolysis index (H) was as follows: $H = 1/A \times [(\Delta Abs_2) - B \times (\Delta Abs_3)]$

Where A is the scaling factor for hemoglobin, B is the correct H measurement for lipemia, and $\Delta Abs_{2,3}$ is the absorbance of the 570-600 and 660-700 nm bichromatic

Figure 1. A disrupted white blood cell (WBC) shows nuclear membrane rupture at the left side and cytoplasm rupture at the supero-temporal side (black arrow). A normal WBC (black arrowhead). Wright Giemsa stain at ×400. All specimens were examined by one pathologist (H.S.M.).

readings, respectively, in relation to the blank absorbances.

Statistical analysis was conducted using SPSS software version 12.0 (IBM Corp., Armonk, NY, USA). We analyzed whether there was a significant difference using the generalized linear regression analysis, and statistical significance was defined as a $p < 0.05$.

Results

In the experiment using a 25-gauge ocutome, the hemolysis indices were 43, 53, 69, 88, 123, and 197 at 0, 800, 1,200, 2,500, 5,000, and 8,000 CPM, respectively. In the experiment using a 27-gauge ocutome, the hemolysis indices were 52, 78, 78, 131, 186, and 375 at 0, 800, 1,200, 2,500, 5,000, and 8,000 CPM, respectively. Both ocutome diameter and cutting rate affected the hemolysis index, and there were significant differences ($p < 0.001$, $p = 0.033$; Fig. 2).

In the experiment using a 25-gauge ocutome, the WBC break rates were 1.67%, 0%, 3.48%, 2.97%, 3.81%, and 1.82% at 0, 800, 1,200, 2,500, 5,000, and 8,000 CPM, respectively. In addition, in the collected blood using a 27-gauge, the WBC break rate was 0.87%, 0.92%, 1.80%, 1.50%, 2.52%, and 8% at 0, 800, 1,200, 2,500, 5,000, and 8,000 CPM, respectively. Although this ratio did not show a significant difference depending on the diameter of the ocutome

Figure 2. Correlation between hemolysis index and ocutome cutting rate. In the experiment using a 25-gauge (G) ocutome, the hemolysis indices were 43, 53, 69, 88, 123, and 197 at 0, 800, 1,200, 2,500, 5,000, and 8,000 cuts per minute (CPM), respectively (gray line). In the experiment using a 27-G ocutome, the hemolysis indices were 52, 78, 78, 131, 186, and 375 at 0, 800, 1,200, 2,500, 5,000, and 8,000 CPM, respectively (black line).

($p = 0.763$), the difference in WBC break rate following an increase in cutting rate was significant ($p = 0.030$; Fig. 3). Fig. 4 shows RBC and WBC changes according to ocutome diameter and cutting rate in the pathologic examination with Wright Giemsa stain.

Figure 4. (A) Specimen obtained with a 25-gauge ocutome at a cutting rate of 0 cuts per minute (CPM) with well-preserved red blood cells (RBCs) and white blood cells (WBCs) with good cellularity. (B) Specimen obtained with a 25-gauge ocutome at a cutting rate of 800 CPM with well-preserved RBCs and WBCs with good cellularity. (C) Specimen obtained with a 25-gauge ocutome at a cutting rate of 8,000 CPM. Some RBCs were destroyed and WBCs were normal. (D) Specimen obtained with a 27-gauge ocutome at a cutting rate of 8,000 CPM. Most RBCs were destroyed. The WBC that appears to be a macrophage is damaged (yellow arrow), and another WBC appears to be normal (black arrow). Wright Giemsa stain at $\times 400$. All specimens were examined by one pathologist (H.S.M.).

Figure 3. The correlation between white blood cell (WBC) break rate and ocutome cutting rate. In the experiment using a 25-gauge (G) ocutome, the WBC break rates were 1.67%, 0%, 3.48%, 2.97%, 3.81%, and 1.82% at 0, 800, 1,200, 2,500, 5,000, and 8,000 cuts per minute (CPM), respectively (gray line). In the experiment using a 27-G, the WBC break rate was 0.87%, 0.92%, 1.80%, 1.50%, 2.52%, and 8% at 0, 800, 1,200, 2,500, 5,000, and 8,000 CPM, respectively (black line).

Discussion

Diagnostic vitrectomy assists with diagnosis of lymphoma, infectious uveitis, and posterior segment inflammation. As a number of primary intraocular lymphomas involve the central nervous system, early diagnosis is crucial [23]. Evaluation of cell loss in the specimen collection process is essential because the number of cells included in the specimen obtained from diagnostic vitrectomy is small. To increase the yield of diagnostic vitrectomy, parameters including cutting rate, vacuum, duty cycle, ocutome diameter, air infusion, or perfluorocarbon perfusion during vitrectomy need to be controlled.

In this study, we attempted to increase the yield of diagnostic vitrectomy using blood samples by controlling the cutting rate and ocutome diameter among the parameters. As a result, we found that a small-gauge needle affected the hemolysis index but not the WBC break rate, possibly due to plasticity. The size of the RBCs was approximately 7.5–8.7 μm , and the size of the WBCs was approximately 10–15 μm . WBCs are larger than RBCs but have an amorphous cell membrane. Therefore, WBCs are flexible, while RBCs are relatively rigid; thus, RBCs might be more liable destruction in the small lumen.

Previously, Trikha et al. [24] reported the cell viability and diagnostic yield when applying 20-gauge and 25-gauge vitrectomy to cultured human Burkitt lymphoma cells (Raji B-cell lymphoma line). When comparing simple aspiration 20-gauge and 25-gauge vitrectomy based on flow cytometry, there was no difference in cell viability or diagnostic yield of the three cell surface markers CD19, CD45, and kappa light chain. There was no significant difference in results for 600 and 1,500 CPM cutting rates. A WBC loss of approximately 3% was observed for 25-gauge vitrectomy at 1,200 and 2,500 CPM. This was similar to the cell viability of approximately 98% of Trikha et al. [24] in a 25-gauge needle at 1,500 CPM. However, this study measured cell loss at a higher cutting rate, and cell loss greater than 5% was observed at greater than 25-gauge 12,000 CPM. Oshima et al. [16] reported sufficient well-preserved cells for cytopathologic examination at a cutting rate of 800 CPM using a 27-gauge cutter [25]. However, they did not compare findings according to cutting rate or ocutome diameter. To our knowledge, 27-gauge diagnostic

vitrectomy machines with high-speed cutting have not been evaluated with regard to biopsy yield. In this study, WBC loss increased depending on cutting rate in the 27-gauge experiment, and a loss of approximately 8% appeared at 8,000 CPM.

All disrupted cells were identified as macrophages, indicating that larger cells are more vulnerable. Moreover, the density of WBCs may be lower in the vitreous than in the blood. Pretreatment, such as centrifugation, is sometimes required and might decrease the identifiable cell rate. In particular, preservation of large WBCs, such as blast cells, is important in vitreous sampling. If large WBCs are damaged, they can cause errors. As a result, an approximate 8% cell loss may be fatal.

Jiang et al. [26] changed the cutting rate from 0 to 2,500 CPM with low and high aspiration pressures when conducting an experimental vitreous biopsy using a B-cell lymphoma line. They found that cell viability decreased starting at 600 CPM. Ratanapojnart et al. [27] conducted a vitreous biopsy of bacterial, fungal, and leukocyte suspensions at 600 and 1,500 CPM. Although they reported no change in the yield of bacterial specimens at 1,500 CPM, the fungal yield decreased slightly, and leukocyte viability decreased markedly. This was attributed to the size difference in bacteria, fungi, and leukocytes and the more substantial cell damage of the largest cells (leukocytes). Cell viability decreased at a lower cutting rate than that found in the present study likely due to the different clinical settings.

The limitation of this study is the variation in sampling environment due to targeting of blood instead of vitreous. Further studies are needed to investigate and compare 25-gauge versus 27-gauge vitrectomy in human vitreous or in an animal model. As conclusions have been drawn using a sample from only one patient without considering the hematologic characteristics of the individual, there might be errors, and there is uncertainty regarding generalization to a larger population. Moreover, because suction was manually conducted, more data must be collected with sampling suction under constant pressure.

In summary, as a result of tests using blood samples, a smaller gauge and higher CPM increased the hemolysis index. The small-gauge ocutome did not increase the WBC break rate, but the WBC break rate was higher at higher cutting rates. These results suggest that, when collecting a vitreous specimen by diagnostic vitrectomy, ocutome diameter is

not important. However, when collecting vitreous specimen at high CPM, attention is required because the hemolysis index and WBC break rate increase.

Conflicts of Interest

The authors declare no conflicts of interest relevant to this article.

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