Noninvasive Monitoring of Hemoglobin

The Effects of WBC Counts on Measurement

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Abstract

The efficacy of a noninvasive hemoglobin monitoring device (Astrim, Sysmex, Kobe, Japan) was evaluated for healthy volunteers and for patients with hematologic disorders. At the same time, the effects of WBC counts on noninvasive monitoring were studied by clinical evaluation and in ex vivo experiments. The hemoglobin levels determined by the device (Ast-Hb) and a conventional analyzer (T-Hb) were compared. The coefficient of correlation between findings with the Ast-Hb and the T-Hb for healthy volunteers was $r = 0.626$, whereas that for patients with hematologic disorders was $r = 0.762$. A comparison of the ratios of measurement errors in hemoglobin levels by Ast-Hb and T-Hb indicated that the number of WBCs had no effect on hemoglobin monitoring. Moreover, ex vivo studies using isolated WBCs and an optical model that imitates blood vessels and tissue in human fingers confirmed these results. Therefore, this new hemoglobin monitoring device can be expected to be useful for continuous hemoglobin monitoring.

Noninvasive monitoring using near-infrared radiation, such as that used for monitoring the oxygen saturation rate, has come into extensive clinical use.1–4 In 1999, Sysmex, Kobe, Japan, developed a device (Astrim) to determine hemoglobin levels by using the principle of near-infrared spectroscopy in combination with analysis of optical images taken by a charge-coupled device camera located at the opposite side of light sources.5 As shown in Figure 1, the Astrim first detects images of vessels located at the proximal interphalangeal joints, after which the absorption pattern of near-infrared radiation is analyzed to calculate hemoglobin levels. We studied the usefulness of this device as a medical instrument for patients with hematologic disorders and found that the Astrim could detect hemoglobin levels of patients in a manner similar to the detection of such levels in healthy volunteers and that the hemoglobin determination was not affected by the numbers of WBCs or platelets.

Materials and Methods

Subjects

Healthy Volunteers

Ninety-seven healthy volunteers, 59 men and 38 women employed at the Hyogo Red Cross Blood Center, Kobe, Japan, were registered after their written and signed informed consent had been obtained. For the within-run precision study, 7 additional adult volunteers, 5 men and 2 women, were registered in the same manner.
Patients With Hematologic Disorders

Forty-nine patients from Kakogawa Municipal Hospital, Kakogawa, Japan (31 men and 18 women), with various hematologic disorders and hemoglobin levels ranging from 6.7 to 18.4 g/dL (67-184 g/L) were registered after their written and signed informed consent had been obtained. This study was carried out with the approval of the institutional review boards of the Blood Center and Kakogawa Municipal Hospital.

The number of WBCs ranged from 1,400 to 178,700/µL (1.4-178.7 × 10⁹/L) and that of platelets from 14 to 1,131 × 10³/µL (14-1,131 × 10⁹/L). The patients comprised 10 with chronic myelogenous leukemia, 4 with polycythemia vera, 4 with essential thrombocythemia, 3 with other chronic myeloproliferative disorders, 4 with chronic lymphocytic leukemia, 6 with myelodysplastic syndromes, 8 with malignant lymphoma, 4 with multiple myeloma, 1 with hypogammaglobulinemia with miscellaneous causes, 2 with idiopathic thrombocytopenic purpura, and 1 each with aplastic anemia, paroxysmal nocturnal hemoglobinuria, and pure red cell aplasia.

Hemoglobin Measurements

Volunteers and patients were kept at rest for 5 minutes in the sitting position at room temperature before measurement with the Astrim. The middle finger was placed on the detection probe of the Astrim, and hemoglobin levels were determined automatically (Ast-Hb). The results for 3 volunteers and 2 patients were excluded from analysis because their data were considered less reliable, as indicated with an asterisk automatically printed on the data sheet, owing to low finger temperature.

EDTA-anticoagulated blood was obtained on the same day, and true hemoglobin levels (T-Hb) were determined with an SE-9000 (Sysmex). Quality control of the SE-9000 was carried out with the SE-CHECK (Sysmex) every day, resulting in coefficients of variation of 0.4% for hemoglobin values, 2.0% for WBC counts, and 1.8% for platelet counts. External quality management was performed by the Japan Medical Association, the Japanese Committee for Clinical Laboratory Standards, and Sysmex.

The Ast-Hb and T-Hb values for each subject were compared for coefficient analysis, and a ratio of error obtained with the following formula was calculated for statistical comparison:

\[
\text{Ratio of error} = \frac{\text{T-Hb} - \text{Ast-Hb}}{\text{T-Hb}} \times 100
\]

Ex Vivo Study of the Effect of the Number of WBCs on Hemoglobin Detection

A heparinized blood sample was obtained from a healthy donor, and mononuclear (MNCs) and polymorphonuclear cells (PMNs) separated by Monopoly Resolving Medium (Dainippon Pharmaceutical, Osaka, Japan) were washed and suspended in phosphate-buffered saline. The respective purity of MNC and PMN fractions was more than 95% and more than 91%. RBCs also were washed with phosphate-buffered saline after WBC separation. WBCs were mixed with RBCs to obtain a predetermined number of WBCs and T-Hb levels of 7.6 to 10.4 g/dL (76-104 g/L). Ast-Hb levels were measured after the samples were placed in the optical model made of
polyacetal resin, which imitates blood vessels and tissue in human fingers. Polyacetal resin has optical properties similar to those of human tissue. Four samples each were prepared of RBCs with MNCs added, with PMNs added, and without WBCs added, and the ratios of error were compared.

Statistics

Statistical analysis was performed with the StatView 5.0 package (SAS Institute, Cary, NC). Correlation analysis used the Pearson correlation test and the Fisher Z transformation; the ratios of error were compared with the Wilcoxon rank sum test.

Results

Within-Run Precision Study

Table 1 shows the results of hemoglobin measurements by the Astrim for 7 volunteers. The measurements were repeated 10 times. The coefficients of variation ranged from 0.65% to 3.34%.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Mean, g/dL (g/L)</th>
<th>SD, g/dL (g/L)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.31 (153)</td>
<td>0.511 (5)</td>
<td>3.34</td>
</tr>
<tr>
<td>2</td>
<td>14.93 (148)</td>
<td>0.263 (3)</td>
<td>1.76</td>
</tr>
<tr>
<td>3</td>
<td>16.99 (170)</td>
<td>0.110 (1)</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>16.45 (165)</td>
<td>0.127 (1)</td>
<td>0.77</td>
</tr>
<tr>
<td>5</td>
<td>15.16 (152)</td>
<td>0.252 (3)</td>
<td>2.03</td>
</tr>
<tr>
<td>6</td>
<td>12.43 (124)</td>
<td>0.392 (4)</td>
<td>3.15</td>
</tr>
<tr>
<td>7</td>
<td>13.08 (131)</td>
<td>0.310 (3)</td>
<td>2.37</td>
</tr>
</tbody>
</table>

* For product information, see the text.

Plotting of the ratio of error for each of the patients against the number of WBCs (Figure 4) demonstrated that there was no correlation between them. The patients were divided into 3 groups according to the number of WBCs, ie, 17 patients with more than 10,000/µL (>10.0 × 10⁹/L), 17 patients with 4,000 to 10,000/µL (4.0-10.0 × 10⁹/L), and 15 patients with fewer than 4,000/µL (<4.0 × 10⁹/L). The ratios of error for these groups were compared, but no significant difference was observed for any combination.

The effect of the number of platelets also was examined in a manner similar to that used for WBCs, and no significant correlation was detected (Figure 5).

Ex Vivo Study Using an Optical Model

The ratio of error of each sample is shown in Figure 6 as a function of the number of WBCs, showing that there was no significant relationship between them (r = 0.23). In
addition, there was no significant difference in the ratio of error for any combination of RBCs with MNCs added, with PMNs added, and without WBCs added.

**Discussion**

Near-infrared radiation is characterized by high permeability of histologic tissues and can detect changes in the concentration of oxyhemoglobin and deoxyhemoglobin in tissue based on differential absorption of multiple wavelengths. Near-infrared spectroscopy is used clinically to monitor the oxygenation state of hemoglobin. When the Astrim is used, 3 wavelengths of 660, 805, and 880 nm are used to detect images of vessels (Figure 1) and to calculate hemoglobin levels based on the absorption of each wavelength.

We evaluated the efficacy of Astrim for healthy volunteers and patients with hematologic disorders. Although the relationship between Ast-Hb and T-Hb in the volunteer and patient groups was statistically significant, the $r$ values obtained seem to be insufficient for an accurate evaluation of patients with anemia. The specificity and false-positive rate were calculated and receiver operating characteristic curves drawn for male and female subjects, including volunteers and patients with hematologic disorders (Figure 7). Anemia was defined in accordance with the World Health Organization criteria as a hemoglobin level of less than 13 g/dL (<130 g/L) for male and less than 12 g/dL (<12 g/L) for female subjects. The results indicate that the efficacy of the Astrim at this level of precision is clinically restricted. However, because within-run precision results were quite good (Table 1), the Astrim can be expected to be useful for sequential monitoring of hemoglobin levels. Patients requiring sequential monitoring often have some inflammatory complications.
and high WBC counts. Therefore, we checked the effects of the number of WBCs on the determination of hemoglobin levels by means of the Astrim. A clinical evaluation and an ex vivo study were used, and both of these studies indicated that the WBC count did not affect the function of the Astrim.

Kinoshita et al\textsuperscript{9} pointed out major observational errors in the assessment of patients with multiple myeloma and speculated that this error might be related to peripheral circulatory insufficiency due to hypergammaglobulinemia. In our study, however, 5 patients with hypergammaglobulinemia showed ratios of error of –1.7%, 0.1%, 0.5%, 0.9%, and 1.4%, indicating accurate measurements.

The Astrim can be considered useful for continuous hemoglobin monitoring of patients with gastrointestinal bleeding or potential bleeding into pleural or abdominal cavities.

References