Abstract. Background: The relationship between perioperative allogeneic blood transfusion (ABT) and survival following curative surgery for colorectal cancer (CRC) in elderly patients has not been elucidated to date. Patients and Methods: The cases of 108 patients aged 75 years or more who underwent curative surgery for CRC between 2004 and 2011 were retrospectively reviewed. The association between perioperative ABT requirements and other clinicopathological variables was examined. Subsequently, perioperative ABT was compared with other variables concerning overall survival (OS) by univariate and multivariate analyses. Results: Tumor depth, lymph node metastasis and hemoglobin levels were significantly associated with perioperative ABT. Transfused patients had significantly worse OS compared to non-transfused patients. In the multivariate analysis, perioperative ABT (hazard ratio=3.16, 95% confidence interval=1.11-8.98, p=0.031) was the only independent indicator of OS. Conclusion: Perioperative ABT was significantly associated with increased mortality in elderly patients with CRC.

Among patients with colorectal cancer (CRC), 30-75% suffer from preoperative anemia, and they thus require perioperative allogeneic blood transfusion (ABT) (1). The risks of ABT include incompatibility, transmission of infectious agents, coagulopathy and allergic reactions. ABT also has negative effects on the human immune system, a condition termed transfusion-related immune modulation (TRIM). The results of two prior investigations suggested that TRIM is a biological effect associated with the infusion of allogeneic leukocytes and soluble mediators that accumulate in transfused red cells during storage (2, 3). The mechanisms of TRIM include the suppression of cytotoxic cell and monocyte activity, the release of immunosuppressive prostaglandins, the inhibition of interleukin-2 production, and an increase in suppressor T-cell activity (4-7). Several reports have attributed worsened survival rates to the effects of TRIM, initially in patients with CRC (8-10), and subsequently other cancer types, including lung cancer, hepatocellular carcinoma, ampullary cancer, and head and neck carcinoma (11-14).

Over the past decade, it has become apparent that the immune system declines with age, a term known as immunosenescence, which leads to a higher incidence of infections, neoplasia and autoimmune diseases in the elderly (15). Aging of the immune system results in a decline in the absolute number of B-cells and helper and cytotoxic T-cells, and a relative increase in natural killer cells (16). Chronic thymic involution is thought to be one of the major contributing factors to the attenuation of immune system with aging (17). In view of these findings, elderly patients should be recognized as a unique population who are immunologically frail due to the decline of their immune function, and therefore they may be affected more strongly by TRIM than younger individuals.

To the best of our knowledge, the effect of TRIM in patients with immunosenescence has not been investigated, and it is not known how TRIM and immunosenescence act collectively. In the present study, we sought to assess the relationship between perioperative ABT and overall survival (OS) in patients aged 75 years or more, namely immunologically frail patients, who underwent curative surgery for CRC.

Patients and Methods

Patients. Our patient population comprised of 108 patients with pathologically confirmed stage I-III CRC, aged ≥75 years, who underwent curative surgery between January 2004 and December 2011 at the Department of Surgery, Omori Red Cross Hospital in Tokyo, Japan. Patients who underwent a perioperative allogeneic red blood cell (RBC) transfusion formed the transfused group, and those who
did not receive an RBC transfusion formed the non-transfused group. The patients were followed up for 5 years after surgery or until June 30, 2014. Patients who had cancer of other organs were excluded. The medical records of the 108 patients were retrieved from our registry, and we analyzed their clinical and pathological data: age, sex, comorbidities, American Society of Anesthesiologists (ASA) physical status, site of tumor, tumor depth according to the Union for International Cancer Control (18), lymph node involvement, operative time, amount of blood loss during surgery, and hemoglobin (Hb) level at the patient’s initial presentation to our hospital. The OS was also calculated.

Statistical analysis. The correlation between perioperative ABT and the clinical and pathological parameters were assessed by Mann–Whitney U-test for continuous variables, and Pearson Chi-square test or Fisher’s exact test for categorical variables, as appropriate. Cox regression analyses were performed to analyze the OS for the univariate and multivariate analyses. Variables that were significant in the univariate analysis were examined in the multivariate analysis. Kaplan–Meier survival curves and log-rank test were used to compare survival rates. p-Values were two-tailed, and those less than 0.05 were considered significant. All data were analyzed using EZR Ver. 1.24 for Windows software (19).

Results

Twenty-three (21.3%) patients received perioperative ABT. The median follow-up period was 45.5 (range 5.3-60.0) months. Twenty-two (20.2%) out of the 108 patients died during the follow-up period: 13 patients died of CRC, and the remaining nine patients died of other diseases. The clinical and pathological characteristics are shown in Table I.

The proportion of patients with a T3 or T4 tumor depth in the transfused group (95.7%) was significantly higher than that in the non-transfused group (62.4%) (p=0.0017). There was also a significant difference between the proportion of patients

### Table I. The patients’ characteristics at baseline, stratified by perioperative allogeneic blood transfusion.

<table>
<thead>
<tr>
<th></th>
<th>Transfused</th>
<th>Non-transfused</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>81 (76-90)</td>
<td>80 (75-93)</td>
<td>0.21</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13 (56.5)</td>
<td>47 (55.3)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (43.5)</td>
<td>38 (44.7)</td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
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<td></td>
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<tr>
<td>Absent</td>
<td>5 (21.7)</td>
<td>20 (23.5)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>18 (78.3)</td>
<td>65 (76.5)</td>
<td></td>
</tr>
<tr>
<td>ASA physical status</td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>I, II</td>
<td>12 (52.2)</td>
<td>59 (69.4)</td>
<td></td>
</tr>
<tr>
<td>III, IV</td>
<td>11 (47.8)</td>
<td>26 (30.6)</td>
<td></td>
</tr>
<tr>
<td>Site of tumor</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Colon</td>
<td>17 (73.9)</td>
<td>61 (71.8)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>6 (26.1)</td>
<td>24 (28.2)</td>
<td></td>
</tr>
<tr>
<td>Tumor depth</td>
<td></td>
<td></td>
<td>0.0017</td>
</tr>
<tr>
<td>T1, T2</td>
<td>1 (4.3)</td>
<td>32 (37.6)</td>
<td></td>
</tr>
<tr>
<td>T3, T4</td>
<td>22 (95.7)</td>
<td>55 (62.4)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>Absent</td>
<td>12 (52.2)</td>
<td>68 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>11 (47.8)</td>
<td>17 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Operative time (min)</td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>234 (10-2669)</td>
<td>200 (3-920)</td>
<td>0.87</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>8.5 (4.7-13.1)</td>
<td>12.2 (5.7-16.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ASA, American Society of Anesthesiologists.
with lymph node metastasis in the transfused group (47.8%) versus the non-transfused group (20.0%) ($p=0.015$). The median Hb levels in the transfused patients (8.5 g/dl) were significantly lower than that in non-transfused patients (12.2 g/dl) ($p<0.001$). There was no significant difference in any of the other variables between the two groups.

The results of the univariate Cox regression analyses for OS are summarized in Table II. The factors significantly associated with poorer OS were greater tumor depth ($p=0.014$), lymph node metastasis ($p=0.0022$), lower Hb level ($p=0.036$), and receipt of perioperative ABT ($p=0.001$). The multivariate Cox regression analysis showed that the use of perioperative ABT [hazard ratio (HR)=3.16; 95% confidence interval (CI)=1.11-8.98; $p=0.031$] alone was independently associated with OS (Table III). The patients who received perioperative ABT had a significantly worse 5-year OS rate than those without perioperative ABT (46% vs. 83%, $p<0.001$) (Figure 1).

Since deeper tumor depth and lymph node metastasis were risk factors for perioperative ABT (Table I), we performed sub-group analyses of patients with stage I, II and III CRC. In the stage I sub-group, no patients received perioperative ABT (Figure 2A). In the stage II sub-group, the patients who received perioperative ABT had a lower 5-year OS rate (66%) compared to that of those without perioperative ABT (74%), although the difference was not significant ($p=0.18$) (Figure 2B). In patients with stage III disease, the 5-year OS rate was significantly lower in the transfused group (18%) than in the non-transfused group (81%) ($p=0.015$) (Figure 2C).

Because pre-storage leukoreduction (PSLR) was implemented for allogeneic RBC transfusions in January 2007 in Japan, we also divided the 108 patients into four groups based on when they were treated: patients transfused in 2004-2006, those transfused in 2007-2011, non-transfused patients in 2004-2006 and non-transfused patients in 2007-2011, and we compared their OS rates. Among the patients who received perioperative ABT, there was a tendency for an improved 5-year OS rate in those treated in 2007-2011 compared to those treated in 2004-2006 (50% vs. 29%, $p=0.065$) (Figure 3). Among the patients without perioperative ABT, the 5-year OS rates were similar in those treated in 2004-2006 and those treated in 2007-2011 (80% vs. 84%, $p=0.64$) (Figure 3).

Among the patients treated in 2007-2011, those who received perioperative ABT had a significantly lower 5-year OS rate (50%) compared to those who did not (84%, $p=0.0030$) (Figure 3). A similar significant difference was also obtained in the patients treated in 2004-2006 (29% vs. 80%, $p<0.001$) (Figure 3).

Discussion

In this study, the OS rate in the 23 elderly patients of the transfused group was significantly lower than that of the 85 non-transfused patients, and the multivariate analysis revealed that perioperative ABT was an independent prognostic indicator of poorer OS in this group of patients (Table III). These results were consistent with those of a number of previous studies (8-10). Although deeper tumor depth and lymph node metastasis were risk factors for perioperative ABT (Table I), our sub-group analyses of the patients with stage III disease demonstrated that the OS rate in the transfused group was significantly lower than in the non-transfused group. Therefore, it is not the case that patients had worse prognoses simply because they had advanced disease. To the best of our knowledge, this is the first report to investigate the impact of perioperative ABT on the prognosis of elderly patients (who are likely to be in an immunosenescent state) after they have undergone a curative surgery for CRC.

Immunosenescence reportedly contributes to cancer development and progression (15). Compelling evidence supports the notion that several subsets of regulatory T-cells (Tregs) suppress antitumor immunity and promote tumor growth (20). Although the mechanisms of immunosenescence have not been established, emerging data suggest that progressive aging significantly affects the frequency, subset distribution and functional competence of Tregs. Some Treg populations, specifically naturally-occurring Tregs, seem to accumulate with advancing age, whereas inducible Tregs appear to be less available with age compared to those in younger populations (21).
The effects of TRIM are also thought to contribute to cancer evolution (8-10). Although the exact mechanism of TRIM remains to be elucidated, an induction of Tregs by transfusion of stored RBCs has been proposed as one of the mechanisms (22). Allogeneic leukocytes and soluble mediators accumulated in blood products during storage are believed to cause TRIM, and to prevent this effect, PSLR has been implemented in various countries, including Japan (23). In Japan, PSLR was first implemented for platelet products in October 2004, followed by fresh-frozen plasma in March 2006 and RBCs in January 2007. Thus, after January 2007, all allogeneic blood products provided by the Japanese Red Cross Blood Center have been prestorage leuko-reduced products.

The present study's patients transfused in 2007-2011 had a tendency for improved OS compared to those transfused in 2004-2006. This suggests an impact of leukoreduction in preventing TRIM, but further studies are necessary to investigate this possibility. In fact, a 2014 study evaluated the importance of PSLR in preventing alloimmunization to platelet antigens and platelet transfusion refractoriness in...
Japan (24), but this evaluation was not feasible because bedside leukoreduction was routinely performed for patients receiving platelet transfusions in Japan in the period prior to the introduction of PSLR.

Several research groups have reported that pre-treatment of anemia might be an independent prognostic variable for the long-term outcome of patients with CRC (25, 26). Our present findings showed that the Hb level was significantly associated with the requirement for perioperative ABT, as expected, but it was not an independent prognostic factor of OS. On the other hand, perioperative ABT was the only independent predictor of OS in our multivariate analysis. These results indicate that perioperative ABT could be a more authentic and reliable prognostic factor than pre-treatment of anemia.

The major limitations of our study were the small patient cohort at a single Institution, and the study’s retrospective nature. Our sample size was also limited by the fact that elderly patients diagnosed with CRC often suffer from malignancies of other organs; such patients were excluded from this study. Therefore, a future study including a larger study population is required. In addition, although the guidelines issued by the Ministry of Health, Labour and Welfare of Japan for the use of blood products recommend the Hb trigger of 7.0 g/dl for the transfusion of RBC products (27), there is a tendency for individual surgeons/anesthesiologists to use their own Hb trigger, possibly based on their experience; therefore, a uniform Hb trigger was not applied for perioperative blood transfusion in our patients.

In addition, as mentioned above, although the effect of PSLR in preventing TRIM could not be evaluated sufficiently in our series, our results strongly suggest that at least in elderly patients, TRIM was not completely prevented even in those who received PSLR RBC products, i.e. those transfused after January 2007. In Japan, there is an increasing tendency for the use of autologous blood transfusion, which is a measure that can reliably prevent TRIM (28). Several reports have shown the positive impact of autologous blood transfusion compared to ABT in terms of postoperative complications (29), and more recently, the immunological changes caused by allogeneic, but not autologous, blood transfusion have been confirmed (30). Unfortunately, autologous blood transfusion is not performed at our Institution, but our present findings suggest the importance of implementing such blood transfusions as a measure of avoiding ABT, especially in elderly patients who may already be affected by immunosenescence.

In conclusion, the results of the present study clearly demonstrate an inverse correlation between perioperative ABT and OS in elderly patients with CRC. Measures to avoid perioperative ABT, especially in elderly patients who are in an immunosenescent state, should thus be actively considered. Such measures include the preoperative treatment of anemia, which is also recommended in patient blood management guidelines (31), and the use of autologous blood transfusion when feasible.

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References


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