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Leukocyte Depletion in Allogeneic Blood Transfusion Does Not Change the Negative Influence on Survival Following Transthoracic Resection for Esophageal Cancer

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Abstract
Background Perioperative transfusion of allogeneic blood has been hypothesized to have an immunomodulatory effect and influence survival in several cancer types. This study evaluates the association between receipt of leucocyte-depleted and non-depleted allogeneic blood and survival following esophagectomy for cancer.
Methods A retrospective analysis was performed including 291 patients with esophageal cancers who underwent transthoracic en bloc esophagectomy and extended mediastinal lymphadenectomy. Neoadjuvant chemoradiation was administered in 152 (52.2%) patients. Perioperative blood transfusions were quantified and the potential prognostic cutoff for transfused units was calculated according to LeBlanc.
Results The median number of perioperative blood transfusions was 2 (0–24), and 106 patients (36.4%) received no transfusions. Patients with one or less blood transfusion showed a significantly improved survival compared to patients receiving more than one unit ($p<0.009$). In multivariate analysis, blood transfusion categories showed significance ($p<0.015$) next to pT, pN, pM category, and residual tumor categories (R-categories). Separate analysis of 183 patients treated after the mandatory introduction of leukocyte-depleted blood transfusions detected a strong tendency, but no significant difference in survival for patients getting one or less or more than one transfusion ($p=0.056$). Receipt of leukocyte-depleted versus non-depleted units, however, had no influence on survival ($p=0.766$).
Conclusions The need for perioperative allogeneic blood transfusions is significantly associated with poorer survival following resection for esophageal cancer by univariate and multivariate analysis. Our data suggest that the reduction of leukocytes in allogeneic transfusions is not sufficient to overcome the negative influence on survival.

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Keywords Esophageal carcinoma · Allogeneic blood transfusion · Leukocytes-depleted blood transfusion · Survival

Introduction

Since the 1970s, perioperative blood transfusion has been thought to have an immunomodulatory effect. Though beneficial in transplantation surgery, it was postulated to be associated with decreased survival rates in various tumor types including esophageal cancer.[1]

The cause of this phenomenon is still unclear. Some authors assumed decreased survival with perioperative blood transfusion to be rather a reflection of circumstances

necessitating transfusion.[2,3] Others demonstrated a significantly independent relationship by multivariate analysis.[4–6]

Studies exploring underlying mechanisms showed a decreasing number of circulating T lymphocytes with a decreased ratio of helper-to-suppressor T lymphocytes. Natural killer cell function was reduced like the interleukin-2 production, whereas the number of suppressor T lymphocytes and production of prostaglandin-E was increased. In summary, allogeneic blood transfusion decreases T-cell-based immune response, an important mechanism to remove tumor cells from the body. Furthermore, macrophage migration was found to be impaired preventing antigen presentation.[1]

In animal models, allogeneic blood transfusion was associated with increased tumor growth and frequency of metastases.[7] These effects could possibly be abolished through depleting donor blood of leukocytes.[8,9] In patients undergoing colorectal surgery receiving leukocyte-depleted transfusions, postoperative infections decreased significantly despite impaired natural killer cell function.[10]

Previous studies dealing with blood transfusion in esophageal surgery did not deal with leukocyte-depleted transfusions.[2–6,11] After the mandatory introduction in Germany in 2001, pre-storage leukocyte-depleted products are used without exception, with an amount of contaminating leukocytes of less than 1×10^6/unit according to the European consensus.[12]

In this retrospective analysis, we evaluated the prognostic influence of perioperative allogeneic blood transfusions in patients with esophagectomy for esophageal cancer. Comparisons were made between patients treated with leukocyte-depleted versus non-depleted units of blood.

Material and Methods

A retrospective study was performed with 305 patients who underwent esophagectomy between January 1997 and October 2006 in the Department of Visceral and Vascular Surgery, University of Cologne, Germany. To exclude the effects of surgery-related postoperative complications, 14 patients (4.8%) dying within 90 days after the operation were excluded.

From 291 study patients, there were 234 men (80.4%) and 57 women (19.6%) with a median age of 62 years (range, 18.9 to 83.2 years).

Histopathological examination of the resected specimens revealed squamous cell cancer in 137 patients (47.1%), adenocarcinoma in 148 cases (50.9%), and other rare entities in six patients (2.1%).

Because of locally advanced disease, 152 patients (52.2%) received standardized neoadjuvant chemoradiation with cisplatin, 5-fluorouracil, and 36 Gy as described in detail.[13] Four to five weeks after completion of chemoradiation, transthoracic en bloc esophagectomy with two-field lymphadenectomy was performed. Relevant clinical and histopathological data are summarized in Table 1.

To determine the number of perioperative blood transfusions, an observation period of 30 days after operation was chosen. One unit equals approximately 280-ml (250–310 ml) suspension of packed red blood cells.

An Edict of the Paul-Ehrlich-Institute allowed only the use of leukocyte-depleted blood transfusions after October 1, 2001 in Germany. Pre-storage depletion achieves an amount of contaminating leukocytes of less than 1×10^6/unit according to the European consensus.[12]

Statistical Analysis

The median follow-up was 4.9 years (range, 1.1–11 years). All living patients had a follow-up of more than 12 months.

Table 1 Patient Characteristics

Parameter	Number of patients (%)
Median age, 62.0 years (range, 18.9–83.2 years)	
Gender	*n*=291
Male	234 (80.4%)
Female	57 (19.6%)
Histology	*n*=291
Squamous cell cancer	137 (47.1%9
Adenocarcinoma	148 (50.9%)
Others	6 (2.1%)
Neoadjuvant treatment	*n*=291
No	139 (47.8%)
Yes	152 (52.2%)
T category	*n*=291
pT0	27 (9.3%)
pT1	68 (23.4%)
pT2	58 (19.9%)
pT3	136 (46.7%)
pT4	2 (0.7%)
N category	*n*=291
pN0	149 (51.2%)
pN1	142 (48.8%)
M category	*n*=291
cpM0	249 (85.6%)
cpM1	42 (14.4%)
Grading	*n*=291
G1	5 (1.7%)
G2	148 (50.9%)
G3	135 (46.4%)
G4	3 (1%)
R category	*n*=291
R0	276 (94.8%)
R1/2	15 (5.2%)

pT local invasiveness, *pN* lymph node metastases, *c/pM* distant metastases (categories according to UICC), *y* neoadjuvant therapy, *n* number of patients

We analyzed the best cutoff value for number of blood transfusions (ec) as a prognostic variable by simulating the log-rank test for groups defined by (ec < c) and (ec > c) for observed values of the covariate for the entire data set. This tree-based method for prognostic stratification was described by LeBlanc.[14]

Kaplan–Meier plots were used to describe survival distribution.[15] The log-rank test was used to evaluate for survival differences.[16] For multiple comparisons, the Holm–Sidak method was used. In addition, 95% confidence intervals (95% CI) for the different survival curves were calculated. Cox regression analysis was applied to identify independent prognostic variables. The level of significance was set to $p<0.05$.

All statistical tests were performed using the Software Package SPSS for Windows, version 14.0, Chicago, IL, USA.

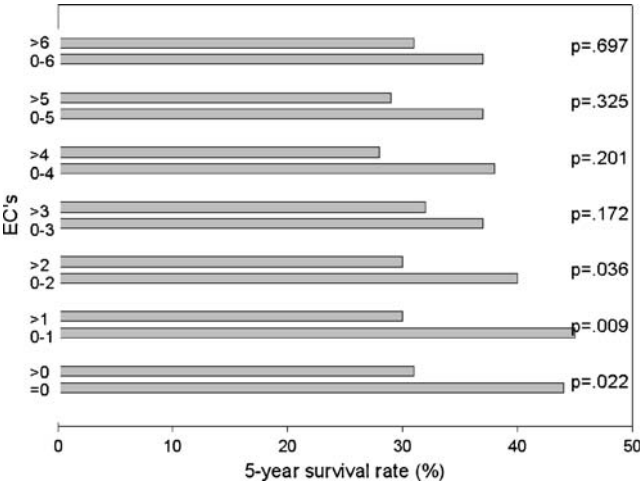

Figure 1 Analysis of the best cutoff value for number of blood transfusions (ec) as a prognostic variable by simulating the log-rank test for groups defined by (ec < c) and (ec > c) for observed values of the covariate for the entire data set.[14]

Results

Transfused Allogeneic Blood Units

Transfusion demand ranged from 0 to 24 U of blood. Median was 2 U and mean value was 3.5±4.5 U. One hundred six patients (36.4%) received no allogeneic blood transfusions, five patients only one unit (1.7%).

There were 108 cases before October 1, 2001, whereas after introduction of leukocyte-depleted blood, 183 patients were analyzed. In the first period, 18 patients (16.7%) were not transfused, and one person received a single unit (0.9%). In the second period, 88 patients (48.1%) were operated without allogeneic blood and four patients with a single unit (2.2%). The median number of blood transfusions decreased significantly from 4 to 1 U ($p<0.001$).

Survival Analysis

The 5-year survival rate for all patients was 35±3%. A significant cutoff value was identified between 0–1 and >1 U of transfused blood (Fig. 1). Five-year survival rates for patients with 0–1 U transfused blood was 46±7% compared to 29±4% for patients with >1 U [median survival 3.52 (0.99–6.04) vs. 2.1 years (1.66–2.54), $p<0.009$] (Fig. 2).

Currently, 126 patients are alive, 158 died, and seven patients were lost to follow-up.

In a subgroup analysis of 183 patients treated after the mandatory introduction of leukocyte-depleted blood transfusions in October 2001, no significant difference in survival ($p=0.056$, Fig. 3) was found for patients getting one or less or more than one transfusion [5-year survival rates, 39±9% compared to 29±7%; median survival was 3.24 (1.51–4.98) vs. 2.25 (1.66–2.84) years].

The comparison of transfused patients before and after October 2001 failed to show a significant difference ($p=0.766$) between non-depleted units (5-year survival, 30±5%) versus leukocyte-depleted units (5-year survival, 30±7%) on survival (Fig. 4) [median survival was 1.96 (1.33–2.60) vs. 2.34 years (1.65–3.03)].

Multivariate Analysis

Cox regression analysis for all patients including pT, pN, c/pM categories, resection categories, histology, neoadjuvant therapy, and blood transfusion categories showed significance for pT [$p<0.002$, HR 2.9 (1.5–5.6) (pT3–pT1)], pN [$p<0.0001$, HR 2.0 (1.4–3.1)], pM [$p<0.011$, HR 1.7 (1.1–2.5)], residual tumor categories (R-categories) [$p<0.005$, HR 2.4 (1.3–4.5)], and blood transfusion categories [$p<0.015$, HR 1.6 (1.1–2.3)].

Discussion

In the present study, patients receiving none or 1 U of allogeneic blood had a significantly better 5-year survival rate than patients getting more than 1 U by univariate analysis. In multivariate analysis, transfusion category remained independently associated with survival like pT, pN, c/pM, and resection categories.

In the literature, several studies dealing with blood transfusion and survival in patients with esophageal carcinoma are published, most of them with mixed histological types.

Langley et al.[4] demonstrated transfusion of more than 3 U to be an independent negative predictor for survival by

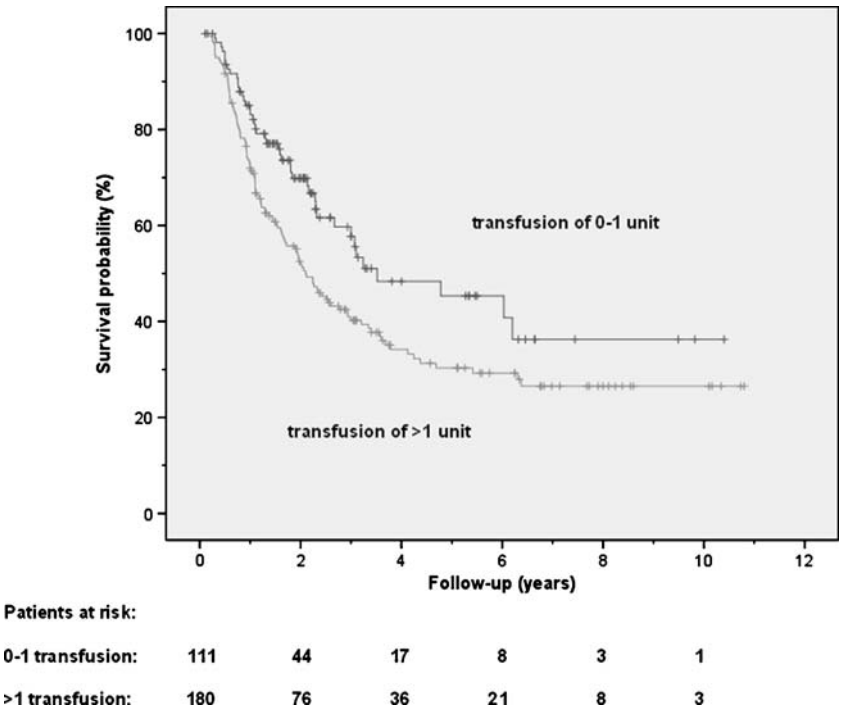

Figure 2 Kaplan–Meier curves based on blood transfusions (≤1 versus >1; cutoff according to LeBlanc, log-rank, $p<0.009$). Overall survival rate at 5 years for all patients was 35±3%.

multivariate analysis. This observation is supported by Tachibana et al.[5] who identified more than 2 U as independent prognostic factor in squamous cell cancer patients. As in our study, they excluded all patients dying within 90 days after the operation to exclude effects of surgery-related postoperative complications. Dresner et al.[6] confirmed transfusion of more than 4 U as independent factor in esophageal cancer patients.

In contrast, Swisher et al.[2] demonstrated blood transfusion of more than 8 U to be associated with decreased long-term survival by univariate analysis. This, however, was due to an increased number of postoperative complications which then eliminated significance in multivariate analysis. The authors suggest that complications that necessitate transfusions were responsible for this observation because no increase in local or distant tumor recurrences were

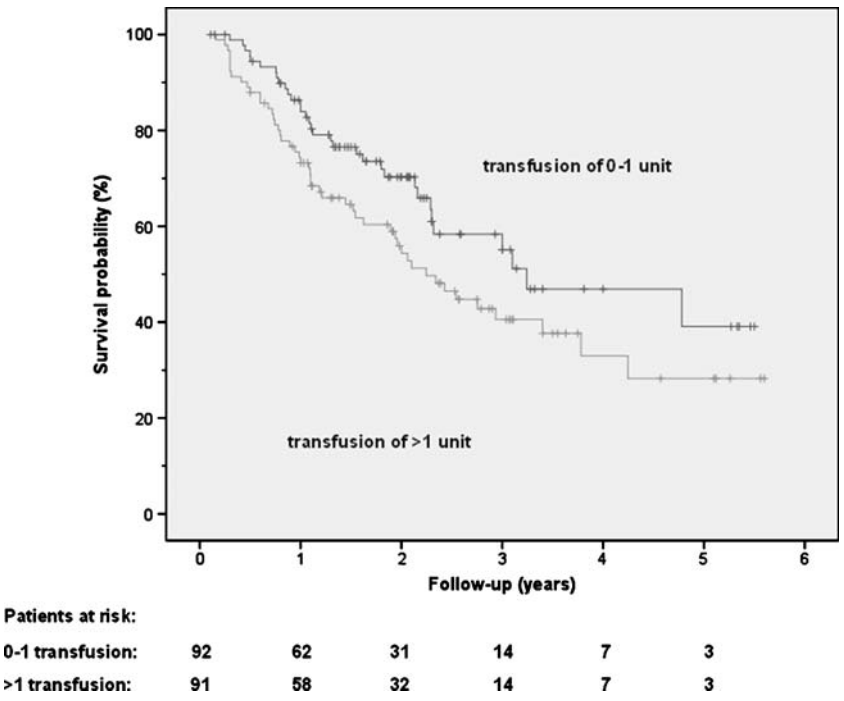

Figure 3 Kaplan–Meier curves based on blood transfusions (≤1 versus >1; cutoff according to LeBlanc) for the subgroup of 183 patients treated after the mandatory introduction of leukocyte-depleted blood transfusions in October 2001. Log-rank failed level of significance ($p=0.056$) for patients getting more than one transfusion (5-year survival, 29±7%) versus ≤1 (5-year survival 39±9%).

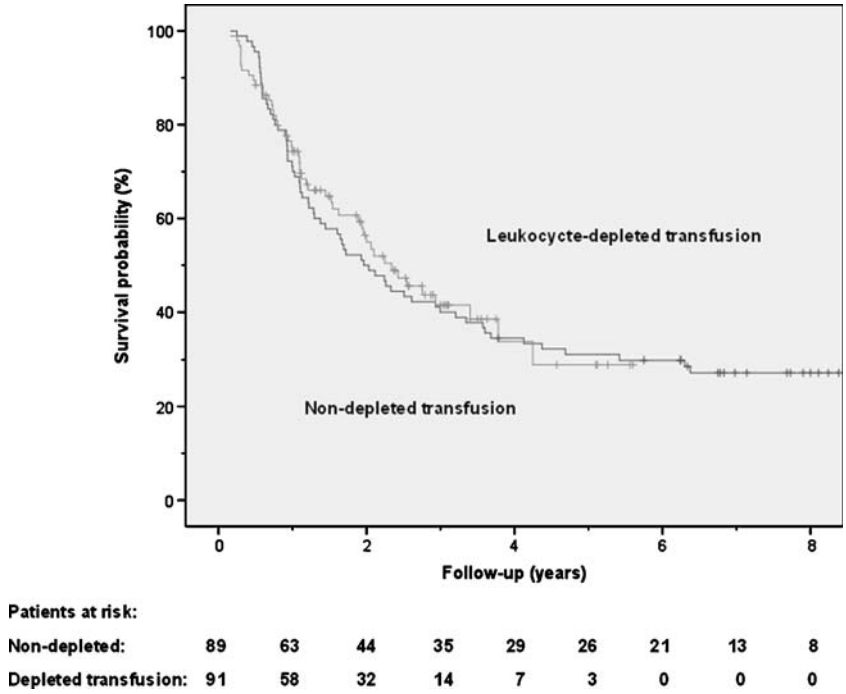

Figure 4 Kaplan–Meier curves for patients receiving non-depleted (5-year survival, 30±5%) versus leukocyte-depleted transfusions (5-year survival, 30±7%), log-rank, $p=0.766$.

identified. Nozoe et al.[3] also found that the occurrence of postoperative complications was a prognostic factor, whereas perioperative allogeneic blood transfusions were not in patients with squamous cell cancer.

Craig et al.[11] identified that blood transfusion is only associated with reduced short-term survival for patients in advanced stage III. They hypothesized that occult micrometastases progressed in this subgroup due to transfusion-induced immunomodulatory effects.

This is in line with a study by Motoyama et al.[17] comparing autologous with allogeneic blood transfusions. Differences were seen in prolonged disease-free survival for patients getting autologous blood, but not for recurrence rates or survival times following recurrence. Takemura et al.[18] demonstrated that patients with nodal involvement and T3/4 tumors had a significantly improved survival after autologous compared to allogeneic transfusion.

Because contaminating donor leukocytes could be responsible for this effect, we sub-analyzed our data for operations pre- and post-introduction of leukocyte-depleted blood transfusions (less than 1×10^6 leukocytes/unit according to the European consensus).[12] The comparison of transfused patients failed to show an association between non-depleted versus leukocyte-depleted units on survival. The different follow-up time periods for these groups represent a potential bias, but analysis of Kaplan–Meier curves showed a similarity within the first years of follow-up. The shorter follow-up in the population of leukocyte-depleted blood transfusion might also be the reason for the strong tendency but missing significance of the amount of transfused units towards reduced survival.

In colorectal surgery, Jensen et al.[19] found after transfusion with whole blood an increase in postoperative infectious complications accompanied by elevated IL-2R and IL-6 levels and decreased lymphocyte proliferation and CD4/CD8 ratio in leukocyte-depleted blood only slight and transient changes similar to non-transfused patients. In comparison between buffy-coat-poor and leukocyte-depleted blood transfusion, differences in wound infections and abscesses could be also noticed; however, the mortality rate was not different,[20] even after 7 years of follow-up in contrast to non-transfused patients.[21]

Also, in colorectal cancer, Houbiers et al.[22] found no difference in disease-free survival, cancer recurrence rate, or overall infections between patients receiving buffy-coat-poor or leukocyte-depleted blood. In contrast to non-transfused patients, both groups had a reduced survival and higher infection rate. The effect of blood transfusion on survival might therefore not be mediated by allogeneic leukocytes alone, but also cellular and humoral components could be able to alter the immune potential.[23]

In summary, the need for perioperative allogeneic blood transfusions is associated with poorer survival following resection by transthoracic en bloc esophagectomy for esophageal carcinomas by univariate and multivariate analysis. Our data suggest that either the reduction of leukocytes is not sufficient or that besides leukocytes other cellular or humoral elements may also influence survival after blood transfusion.

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