Introduction

Blood transfusion (BT) is a common practice in surgical procedures, particularly in cancer surgery, where it is often used to manage surgical bleeding and anemia. However, several clinical studies have reported an increased risk of postoperative infections in patients who received BT, which is attributed to immunosuppressive effects induced by BT. These effects are thought to be mediated by the transfused blood, which may include immunocompromised cells and antibodies that can interfere with the host immune response.

The relationship between BT and postoperative infections continues to be controversial, with some studies showing a significant association, while others do not. The present study aimed to investigate the relationship between BT and postoperative infections in patients undergoing surgery for gastrointestinal cancer.

Methods

An observational study was performed on 152 patients affected by gastrointestinal adenocarcinoma who underwent curative surgery at the Department of Surgery of S. Giovanni Calibita Hospital, Rome, Italy. The study included 76 patients who had their immune function evaluated before and after surgery. The overall postoperative infection rate was 28% for the transfused and 4.6% for the untransfused patients.

The univariate analysis of investigated variables indicated that BT, progressive cancer stage, duration of surgery, and drainage of fluids were associated with infection. The multiple logistic regression analysis confirmed BT (p=0.0028) and advanced cancer stage (p<0.001) as significant risk factors for the postoperative infections. The results of immunological tests showed no significant differences between transfused and untransfused patient groups, after surgery. Comparing pre- and postoperative data from individual patients, an impairment of natural killer (NK) activity was observed in all patients regardless of their transfusional status; the synthesis of interleukin-2 (IL-2) and interferon-γ (IFN-γ) was also decreased respectively in the untrasfused and in the transfused patients.

Interpretation and Conclusions

These results indicate that other factors, beside BT, can induce immunosuppressive effects in these patients and thus increase their susceptibility to postoperative infections.

Key words: blood transfusion, gastrointestinal cancer surgery, infective complications, immunomodulation

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Received February 14, 1997; accepted April 14, 1997.
Hospital, Rome. The study has been approved by the hospital ethical committee.

Sixteen patients were excluded from the study: four patients for preexisting infections and four for previous transfusional therapy; two patients had diagnosis of gastrointestinal lymphoma and one had a recent surgical operation; in five patients curative resection was not possible because of extended carcinoma.

The remaining 136 patients, 68 men (mean age 66) and 68 women (mean age 67) were suitable for the study. All patients received prophylactic perioperative antibiotics, generally a long-acting ticarcillin or cephalosporin, associated with an i.m. aminoglyco-
side. Antibiotics were continued for 24 hours postoperatively.

The following characteristics of the patients and of the operations were collected: anatomic location of tumor (1. stomach, 2. cecum, ascending colon, flexures and transverse colon, 3. descending colon and sigmoid, 4. rectosigmoid and rectum); staging of tumor (Dukes’ classification); operative procedures (1. total or subtotal gastrectomy, 2. right emicolectomy, 3. trans-
verse colectomy, left emicolectomy, sigmoid resection, 4. Hartmann’s operation, 5. anterior rectum resection with co-
stomy and abdominoperineal excision); presence of drains; enteral and parenteral nutrition. Other data collected included: age, gender, time elapsed from the onset of symptoms to admission, hemoglobin level, total white blood cell count (WBC), total serum proteins, duration of surgery.

The transfusions were given during the intraoperative or postoperative period (up to 12 days after operation); the type (1. whole blood or red blood cells, Buffy coat not depleted, +fresh frozen plasma and 2. fresh frozen plasma alone) and unit number were reported.

Attending surgeons were the same for all patients.

Postoperative complications were those that occurred within 20 days of operation or until discharge from the hospital. Postoperative fever was defined as a temperature of more than 38°C for at least three days. Fever alone, without an identified infection, was not recorded.

Infective complications were defined as follows:
- pneumonia infection: fever, leukocytosis, clinical and radiological signs of chest infiltrate;
- urinary tract infection: leukocyturia in sediment with clinical signs and symptoms of UTI leading to treatment with antibi-
otics and/or positive urine culture;
- intra-abdominal or perineal abscess: intraperitoneal or pelvic collection of pus, diagnosed by ultrasonography or spontaneous discharge;
- wound infection: erythema and a purulent exudate from the wound.

The immune status of the last 76 consecutive patients was assessed before and 8-10 days after surgery evaluating the natural killer (NK) activity, the in vitro lymphokine synthesis by peripheral blood mononuclear cells (PBMC) and the in vivo and in vitro synthesis of prostaglandin E2 (PGE2).

Lymphokine synthesis and assay

10⁶ PBMC/mL, isolated by centrifugation on ficoll-hypaque, were cultured in RPMI 1640 supplemented with 20 mM HEPES buffer, penicillin, streptomycin, L-glutamine, sodium bicarbonate and 10% fetal calf serum (complete medium, CM), in pres-
ence of phytohemagglutinin (PHA 2 µg/mL) for 48 h. After centrifugation, the supernatants were separated by centrifugation and stored at –30°C until lymphokine quantification was carried out. The lev-
els of interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) were measured in the culture supernatants by using immunoradiometric assay (Medgenix-Fleurus, Belgium) according to the manufacturer’s instructions.

Cytotoxicity assay

2x10⁶ target cells (K562 erythroleukemia cell line) were incubated for 1h with 50 µCi /H9253 (Amersham International Plc., UK) at 37°C. After washing, the target cells were incubated again for 30 min, washed twice and plated in 96 V-well plates at 10⁴ cells/well. PBMC were added to achieve the effectortarget (E:T) ratios of 50:1, 25:1, 12.5:1. After 18h incubation, the super-
natants were harvested and the /Cr release was measured by a γ-counter.

Prostaglandin production and assay

2.5x10⁶ PBMC/mL were cultured in CM with and without 25 mg/µl of lipopolysaccharides (LPS) (Sigma-Aldrich, Milan, Italy) for 48 h. After centrifugation, the supernatants were sepa-
rated and stored at –30°C.

The serum was separated from the blood, collected in tubes containing indomethacin (1% w/v), and processed within 1h of collection. PGE2 levels were evaluated either in the sera or in the supernatants of unstimulated and stimulated cultures, by using PGE2 (125I) Assay System (Amersham International Plc., UK), according to manufacturer’s instructions. Preliminarily, all sam-
ples were purified according to the method reported by Kelly et al. Briefly, aliquots of serum or culture supernatant were extracted with ethanol-water (4:1), additioned with 1% of glacial acetic acid and applied to an Amprep C18 minicolumn, primed with two column volumes of 10% ethanol and washed with water and exane. The PGE2 fraction was eluted with ethyl acetate and evaporated until dry. Then, the extracted PGE2 was converted into its methyl oximate derivative and stored at –30°C for up to 6 days before analysis.

Statistical analysis

Comparisons of the data were performed by SPSS package on the groups of patients: transfused vs nontransfused and infected vs noninfected.

Univariate analysis was done by Pearson’s chi-square test for categorical variables; Student’s t-test and Kolmogorov-Smirnov (K-S) test were used for continuous variables yielding similar results. Here we report the results obtained by the K-S test; a value of p < 0.05 was considered significant.

A logistic regression analysis (forced entry method) was accomplished to investigate the existence of an independent association between BT and postoperative infection, after adjustment for the effects of confounding variables. All the vari-
bles have been included except those concerning the immune functions as performed on a limited number of patients. The effects of surgery plus BT and those of surgery alone on the immunological variables were evaluated by Wilcoxon matched-pairs test. This compared pre- and postoperative data obtained from individual patients, within the appropriate group.

Results

The incidence of postoperative infections in the various groups of patients is reported in Table 1. Infection developed in 28 of the 136 patients (20.6%). Six patients (21.4%) had wound infections, fifteen (53.6%) abdominal or perineal abscess and seven (25%) urinary infections.

<table>
<thead>
<tr>
<th></th>
<th>Infections</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>All patients</td>
<td>(136)</td>
</tr>
<tr>
<td>Non-transfused</td>
<td>2</td>
</tr>
<tr>
<td>Transfused</td>
<td>26</td>
</tr>
<tr>
<td>Transfused with:</td>
<td></td>
</tr>
<tr>
<td>whole blood or packed RBC + plasma</td>
<td>22</td>
</tr>
<tr>
<td>plasma alone</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1. Incidence of postoperative infection in transfused and non-trafsused patients.
Significant differences in infection rate were found between patients not transfused or transfused by only plasma and those transfused by whole blood or packed RBC and plasma (p = 0.002).

In Tables 2 and 3 data of the variables recorded for the 136 patients are reported.

Comparisons of categorical and continuous characteristics between transfused and untransfused patients (Table 2) evidenced that blood transfusion had a significant association with a low level of Hb, a prolonged operative time and surgical drains.

Since the assignment to transfused and untransfused groups could not be done at random, we compared the two patient groups before surgery and transfusional therapy to ascertain possible differences between them. We found that the Hb level was significantly altered at that time. No differences were identified when the patients were compared for age, sex, serum proteins, white cell count, tumor site and cancer stage.

After surgery, we found a significantly longer operation time and more frequent drains in transfused patients, while no differences in surgical procedures and enteral or parenteral nutrition were observed between the two patient groups.

Comparing the same variables between infected and non-infected patients (Table 3), we found...
Infection, immune function and transfusion after surgery

Table 4. Variables associated with infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted odds ratio</th>
<th>95% confidence bounds</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II, III, vs IV</td>
<td>4.059</td>
<td>1.662-9.912</td>
<td>0.0013</td>
</tr>
<tr>
<td>Duration of surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 90 vs &gt; 90'</td>
<td>1.326</td>
<td>0.273-6.430</td>
<td>0.7249</td>
</tr>
<tr>
<td>Drains</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes/no</td>
<td>1.318</td>
<td>1.185-1.449</td>
<td>0.0203</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes/no</td>
<td>7.955</td>
<td>1.793-35.291</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

Table 5. Immunological variables of nontransfused and transfused patients.

<table>
<thead>
<tr>
<th></th>
<th>Nontransfused</th>
<th>Transfused</th>
<th>p values by K-S test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n. 76)</td>
<td>27 (36%)</td>
<td>49 (64%)</td>
<td></td>
</tr>
<tr>
<td>Before surgery</td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td></td>
</tr>
<tr>
<td>IL-2 IU/mL</td>
<td>18.25 (5.49)</td>
<td>5.82 (0.86)</td>
<td>0.004</td>
</tr>
<tr>
<td>IFN-γ IU/mL</td>
<td>134.8 (16.54)</td>
<td>115.6 (10.20)</td>
<td>0.330</td>
</tr>
<tr>
<td>TNF-α pg/mL</td>
<td>4133 (473.87)</td>
<td>3741 (413.83)</td>
<td>0.417</td>
</tr>
<tr>
<td>NK cytotoxicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E:T 50:1 %</td>
<td>43.7 (3.44)</td>
<td>43.8 (3.10)</td>
<td>0.890</td>
</tr>
<tr>
<td>Serum PGE2 pg/mL</td>
<td>25.4 (4.15)</td>
<td>24.25 (4.06)</td>
<td>0.850</td>
</tr>
<tr>
<td>Spontaneous PGE2 release pg/mL</td>
<td>139.0 (46.62)</td>
<td>60.08 (13.30)</td>
<td>0.054</td>
</tr>
<tr>
<td>LPS induced PGE2 release pg/mL</td>
<td>1040 (159.15)</td>
<td>732 (108.63)</td>
<td>0.102</td>
</tr>
<tr>
<td>After surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2 IU/mL</td>
<td>6.85 (1.55)</td>
<td>6.91 (1.53)</td>
<td>0.550</td>
</tr>
<tr>
<td>IFN-γ IU/mL</td>
<td>109.2 (13.27)</td>
<td>83.6 (13.91)</td>
<td>0.065</td>
</tr>
<tr>
<td>TNF-α pg/mL</td>
<td>4540 (560.99)</td>
<td>3614 (397.41)</td>
<td>0.521</td>
</tr>
<tr>
<td>NK cytotoxicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E:T 50:1 %</td>
<td>34.9 (3.69)</td>
<td>29.32 (2.65)</td>
<td>0.438</td>
</tr>
<tr>
<td>Serum PGE2 pg/mL</td>
<td>32.2 (6.02)</td>
<td>38.2 (5.80)</td>
<td>0.499</td>
</tr>
<tr>
<td>Spontaneous PGE2 release pg/mL</td>
<td>147.7 (62.23)</td>
<td>140.5 (39.31)</td>
<td>0.919</td>
</tr>
<tr>
<td>LPS induced PGE2 release pg/mL</td>
<td>873 (154.14)</td>
<td>756 (101.74)</td>
<td>0.514</td>
</tr>
</tbody>
</table>

* T vs NT groups.

In our data set, duration of surgery and the presence of drains were significantly associated with both blood transfusions and postoperative infections. These variables were therefore identified as true confounders. Using a multiple logistic regression model, which included the confounding variables and the blood transfusion as covariates, it was observed that BT and progressive cancer stage were significantly associated with infections (respectively, p = 0.028 and p = 0.0001).

As regarding the immunoreactivity of the patients, immunological tests were carried out in 76 patients (49 transfused and 27 untransfused), either before or after surgery, in order to verify a possible impairment of their immune responses following surgery and transfusional therapy. The results are reported in Table 5.

In our patients, allogeneic BT was associated with a postoperative infection rate of 28%, compared with 4.6% of the untransfused patients. Similar results in transfused and non-transfused patients or those transfused with autologous blood were obtained by others, while some investigators failed to detect an association of allogeneic BT with infection.

The univariate analysis of the investigated variables indicates a significant association of BT, cancer stage, presence of drains and duration of surgery with infections.

After adjustment for the effects of confounding variables, that in our data set were the duration of surgery and the drains, BT and progressive cancer stage continued to be independently associated with postoperative infections.

The significant association of periooperative BT with infections strongly suggests a causal relationship between the two events, according to the hypothesis that allogeneic BT induces a non specific immunodepression which would favor postoperative infective complications. The mechanisms by which BT may cause immunodepression concern cell-mediated immunity and the function of

blood transfusions, a progressive cancer stage, a longer duration of surgery and drains, significantly associated with infections.

Unadjusted odds ratio for risk of infection are reported in Table 4.
macrophages, decreasing their migration toward chemotactic stimuli and/or inducing an increased production of PGE₂. Accordingly, we investigated the synthesis of lymphokines, the NK cytotoxic activity and the production of PGE₂.

Limitations of our study design are that BT cannot be given in blinded crossover fashion for medico-ethical reasons. However, we analyzed separately transfused and non transfused patients, before surgery and any transfusional therapy, to assess the homogeneity of the two groups. As seen, pre-operative clinical and laboratory data and immune responses were similar in the two patient groups, with the exception of the Hb level and IL-2 synthesis by PBMC which were both lower in the transfused patients. The results regarding the Hb concentration can be reasonably explained: these patients needed perioperative transfusion; the synthesis of IL-2, significantly lower in transfused patients, suggests an impaired immunoresponsiveness prior to the operation and BT that could favor postoperative infections.

After surgery, the two groups of patients, transfused and non transfused, did not show significant differences in their immune responses. We also focused on a possible involvement of the PGE₂ in the hypothetical effect of allogeneic BT. We observed that the in vivo and in vitro synthesis of PGE₂ did not significantly rise after surgery, regardless of whether the patients were transfused or not. This indicates that if changes in the prostaglandin metabolism occur in neoplastic patients, as we also observed in respect to healthy subjects, they are not due to BT, and are present in all patients.

Our immunological results are in line with those of some reports and in contrast to the findings of others. Also in animal models, different changes in the immunoresponsiveness of transfused hosts have been described.

Given the diversity of methodology used to assess the patient immune responses, it is very difficult to make comparisons with the reported data and to explain the discrepancies with our results.

In summary, this study showed a significant association of BT and advanced cancer stage with postoperative infections, also after adjustment for the effects of the variables we analysed, while clear alterations of immune responses in transfused patients, as compared to untransfused, were absent. For this reason, we agree with Vamvakas et al. who believe that this relationship could be due to other not analyzed or unknown confounders. The results we obtained by comparing pre- and postoperative data from anyone patient, seem to support such interpretation, as we observed decreased NK cytotoxicity and IFN-γ production in all patients, irrespective of their transfusional status and decreased IL-2 synthesis only in non transfused patients. This suggests that anesthesia, operative trauma or blood loss play a role in inducing immunosuppressive effects.

On the whole, these results, while not excluding, do not support the notion that BTs are the only factor responsible for an immunosuppressive condition that might lead to infection or to a worse outcome in these patients.

References

Infection, immune function and transfusion after surgery

419-25


