AUTOLOGOUS AND ALLOGENEIC BLOOD TRANSFUSIONS IN COLORECTAL CANCER

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CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

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Autologous and allogeneic blood transfusions in colorectal cancer /
Olivier Robert Christoffel Busch. - [S.l. : s.n.]. - Ill.
Thesis Erasmus Universiteit Rotterdam.
- With ref. - With summary in Dutch.
ISBN 90-9008282-4
Subject headings: Autologous blood transfusion / colorectal cancer.

Printed by Haveka B.V., Alblasserdam.

AUTOLOGOUS AND ALLOGENEIC BLOOD TRANSFUSIONS

IN COLORECTAL CANCER

AUTOLOGE EN ALLOGENE BLOEDTRANSFUSIES

BIJ HET COLORECTAAL CARCINOOM

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. P.W.C. Akkermans, M.A.
en volgens besluit van het college voor promoties.
De openbare verdediging zal plaatsvinden op
donderdag 8 juni 1995 om 13.30 uur

door

Olivier Robert Christoffel Busch

geboren te Roermond

Promotiecommissie

Promotor:	Prof. Dr. J. Jeekel
Co-promotor:	Dr. R.L. Marquet
Overige leden:	Prof. Dr. B. Löwenberg Prof. Dr. G. Stoter Prof. Dr. C.J.H. van de Velde

The investigations presented in this thesis were performed at the Department of Surgery of the University Hospital Rotterdam - Dijkzigt and the Laboratory for Experimental Surgery of the Erasmus University Rotterdam, The Netherlands. The Department of Epidemiology and Biostatistics of the Erasmus University Rotterdam participated.

The study was financially supported by a grant from the Dutch Cancer Society (Koningin Wilhemina Fonds, Nö: 86-13) and a grant from the Dutch Foundation for Preventive Medicine (Praeventiefonds, Nö: 28-1953).

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Contents

CHAPTER 1

GENERAL INTRODUCTION

Since blood transfusion became a therapeutical option in patient care, the surgical possibilities have increased tremendously. Since the problems of anticoagulation and blood group typing were largely resolved, blood transfusions were in only a minority of cases directly lethal. However, it was estimated that still up to 20% of the blood transfusions induces a sort of adverse side-effect.[1] Because the most important side-effects are a result of the transmission of infections and the induction of immunological reactions, these are further discussed.

Blood transfusion-induced infection

Numerous infections can be transmitted by blood transfusions. In developing countries bacterial and parasitic infections, such as Plasmodium (malaria), Trypanosoma cruzi (Chagas' disease), and Treponema Pallidum (syphilis) may occur. In the Western World the most important infections that may be transmitted by blood transfusions are viral infections. Examples of these are the hepatitis B and C virus,[1] human immunodeficiency virus (type 1 and type 2),[2] cytomegalovirus,[3] Epstein-Barr virus, and human T cell leukemia virus.[4] Some of these infectious agents have great clinical impact and therefore blood donors should be routinely screened for them.[5] Since in The Netherlands all donor blood is tested for human immunodeficiency virus (HIV) and hepatitis B and C virus, the risk of hepatitis and AIDS (acquired immune deficiency syndrome) is reduced remarkably,[6] but the transmission of these viruses by blood transfusions is still not zero. The chance on transmission of HIV in The Netherlands is estimated to be 1 in 600,000 transfusions.

Blood transfusion-induced immunosuppression

A blood transfusion is a transplantation of allogeneic tissue in liquid form. Looking to blood transfusions in this way makes it easier to understand why blood transfusions may evoke immunologic reactions. If the recipient's immune system responds to the antigens present on transfused erythrocytes, these red cells will be hemolysed. This hemolytic transfusion reaction occurs mainly as a result of blood group incompatibility, and can be reduced to a minimum by crossmatching. Whenever an allogeneic reaction occurs due to sensitization against other components in the blood (leukocytes, platelets, plasma proteins), a non-hemolytic transfusion reaction occurs. The initial clinical manifestation of this reaction is fever and is usually mild.

Patients who are on the waiting list for a kidney transplantation often need multiple blood transfusions due to renal failure. While investigating whether the induction of antibodies against HLA-antigens by allogeneic blood transfusions would cause an impaired kidney-

graft survival, Opelz et al.[7] found that besides this alloimmunization also immunosuppression occurred. It was found that kidney-graft survival in patients receiving transfusions prior to surgery was as much as 20% better as compared to those patients who did not receive transfusions. Although its exact mechanism is still unclear, the immunosuppressive effect of blood transfusions has for many years been well established in renal transplantation.[8] However, after cyclosporine A was introduced as a very potent immunosuppressive drug the graft survival of patients who did not receive transfusions was improved considerably, and therefore transfusion-induced immunosuppression became less demonstrable.

During the last decades many immunological changes have been held responsible for the immunosuppressive effect of blood transfusions. It has been found that after blood transfusions lymphocyte responsiveness was reduced,[9] T-suppressor lymphocytes were increased,[10] cutaneous delayed hypersensitivity was reduced,[11,12] macrophage activity was impaired,[13] natural killer cell activity was depressed,[14] etc. It also has been found that non-cellular mechanisms might be responsible for transfusion-induced immunosuppression. Prostaglandin E_2 release may be increased after transfusions.[15] An increased level of prostaglandin E_2 is immunosuppressive due to inhibition of interleukin 2, which decreases the function of T cells.[16,17] This influence can be mediated directly by interleukin 2 or indirectly via interferon-γ.[18]

Although all the aforementioned mechanisms may have a role in the immunomodulatory effects of blood transfusions,[19,20] it is important to realize that these findings cannot be automatically extrapolated to tumor biology.[21] For example, HLA-antigens are of great importance in the allograft reaction, and there are indications that the transfusion effect in renal transplantation is dependent on a certain degree of HLA matching and mismatching between blood donor and recipient.[22] However, HLA-antigens are not likely to be involved in the syngeneic relationship between tumor and host.

Blood transfusions and cancer prognosis

Gantt[23] speculated on a possible similarity between tumor and transplantation antigens and suggested in 1981, that blood transfusion-induced immunosuppression might have a detrimental effect on the prognosis of patients operated for solid malignancies. Transfusion-induced immunosuppression can alter tumor growth only if there exists a relationship between tumor growth and the immune system. Although most human tumors lack immunogenicity, it is kwown that non-specific immune responses may have antitumor effects. Cytokines, such as tumor necrosis factor alfa, interleukin-2 and interferon-γ have antitumor effects. Therefore, the activated T cells and macrophages may play a role in the immune response against cancer cells. Rosenberg et al.[24] showed that interleukin-2 therapy had an effect on tumor growth of melanoma and renal cell cancer. Also natural

killer cells have shown to have antitumor effect in vitro. It is conceivable, that blood transfusions mediate tumor growth by altering the non-specific immune response. Additionally, it is found that in colorectal cancer patinets immunotherapy may be a clinical possibility for treatment. The study by Moertel et al.[25] and the study by Laurie et al.[26] demonstrated that there is some therapeutical effect of the immunomodulator Levamisole in combination with Fluorouracil in patients operated for colorectal cancer. Immunotherapy using lymphokine activated killer cells is believed to be effective in patients with advanced colorectal cancer.[27] It is also found that adjuvant therapy with monoclonal antibodies extends life and prolongs remission in patients with colorectal cancer of Dukes' C stage.[28] The increased survival after these treatments suggests a relationship between colorectal cancer prognosis and immune functions. Therefore, the statement that blood transfusions modulate tumor growth has stimulated many investigators to unravel this postulation in either experimental or clinical studies.

Experimental studies

Because of their ability to control for factors such as tumor load, transfusion policy, and surgical extent, experimental studies are the best way to explore a direct effect of blood transfusions on tumor growth. However, the animal studies on tumor growth and blood transfusions showed a great variety in results. Francis et al.[29] found that tumor growth was potentiated by allogeneic blood transfusions in a rat model. However, in another rat model an inhibitory effect of allogeneic transfusions on pulmonary metastases was demonstrated by Jeekel et al.[30], whereas there was no effect on subcutaneously implanted tumors in the same rat strain. Oikawa et al.[31] also did not find an effect on subcutaneously implanted tumors. In these models blood transfusions were given before tumor inoculation. It was also found in rat studies that allogeneic blood transfusions increased tumor growth only when the transfusion was given after tumor inoculation.[32,33] In a mouse tumor model it was found that blood transfusions had a tumor promoting effect when given prior to tumor inoculation.[34] However, Zeller et al.[35] found contradictory results in several other mouse models. Blood transfusions given after tumor inoculation in mice has also demonstrated to have stimulatory effects on tumor growth.[36] All these different animal studies using different models thus have shown an inhibitory, a tumor promoting, or no effect of blood transfusions. Therefore, the presence of a blood transfusion effect on tumor growth seems to be dependent on the kind of tumor, the route of tumor inoculation, and the animal species used.[37] To investigate which blood component could be responsible for a tumor promoting effect animal studies were performed in our laboratory. It was shown that a tumor promoting effect of allogeneic blood transfusions was evoked by the transfusion of erythrocytes, leukocytes and of whole blood whereas transfusion of plasma had no effect on tumor growth.[38]

The finding that some animal models showed a tumor promoting effect of blood transfusions suggests that there indeed might be a clinical situation in which there is an

effect of blood transfusions on tumor recurrence. However, it should be realized that blood transfusions are always given for a particular reason. One of the most common reasons for transfusion is a certain amount of blood loss. It is found that blood loss itself may have immunosuppressive effects. It is also shown by experiments from our own laboratory that blood loss affected tumor growth, irrespective whether transfusions were given or not.[39,40] The amount of blood loss during surgery could be related to the extend of the surgical trauma. Also the extend of the surgical trauma itself can have immunomodulatory effects, showing that the complexity of the immunological changes after an operation includes the effects of blood transfusions, blood loss and surgery. If blood loss during surgery is comparable to the donation of blood this might have clinical consequences when predeposit autologous blood donation is use to reduce the exposure to allogeneic transfusions (see further in this chapter).

Clinical studies

The first retrospective study on the effect of blood transfusions and colorectal cancer prognosis was reported by Burrows and Tartter.[41] The disease-free survival rate in transfused patients was significantly worse than in patients who did not receive transfusions; disease-free survival at 5 years was 51% and 84%, respectively. After this initial report many clinical studies were performed in a variety of tumors. Most of these studies had a retrospective design and dealt with colorectal cancer. Several reports reviewed over 30 of these studies.[42,43,44,45,46,47] A significant adverse effect of blood transfusions was found in a little more than 50% of the studies whereas the other studies showed no significant effect and even in one study blood transfusions even had a beneficial effect on prognosis.[48] In most of these papers multivariate analysis was used to control for various prognostic factors. In some studies, the significant difference observed in univariate analysis, disappeared when multivariate analysis allowing for various factors was performed.[49,50]

Clinical studies reporting a transfusion effect on prognosis cannot differentiate between a causal or an indirect relationship. The latter means that the outcomes were biased by the selection of patients, since patients were transfused either by nature of their disease or by the kind of operation performed. Clearly, multivariate analysis cannot control for factors which are either unknown or difficult to quantify. Factors such as the preoperative condition and the host defence of a patient can only partly be represented by determinants such as age, hemoglobin level, tumor stage, and immunologic parameters. Nevertheless, such factors might influence the recovery and the prognosis of cancer patients as well. Therefore the effect of blood transfusions might be an epiphenomenon, meaning that it is only an indicator of prognostic factors, which could not be adjusted for. Therefore, performing multivariate analysis in the present circumstances cannot reveal whether blood transfusions are causative of a worse prognosis. It can only demonstrate whether they are of additional prognostic value when other known prognostic factors are taken into account.

Obviously, the need for transfusions is related to perioperative blood loss and, therefore, with difficulties during operation, which might be related to the tumor or the surgeon.[51] The extent of a resection and thereby the surgical trauma, reflected by an increased need for transfusions, could be of importance for the patients recovery and also for the induced immunosuppression, which might affect the prognosis. Only a few studies included the extent of operation by allowing for duration of surgery. Parrott et al.[52] found that duration of surgery was significantly related to blood transfusions. However, there was no relationship demonstrated between duration of surgery and prognosis, whereas blood transfusion was a strong predictor of recurrence. Another surrogate marker of surgical extent is blood loss. This factor, however, is strongly correlated with blood transfusions. Therefore blood transfusions and blood loss are difficult to evaluate separately in clinical studies, but as was shown in animal experiments the factor blood loss might be relevant for perioperative immunosuppression.

In some studies, including the one from our hospital,[53] it was suggested that the transfusion effect might be due to an increased amount of patients with rectal tumors in the transfused group, who apparently have a worse prognosis. Controlling for tumor location in multivariate analysis, however, it was found that blood transfusion was an independent factor. In addition, there are several reports which dealt only with colonic cancer which also found a detrimental effect of blood transfusions.[54,55]

Voogt et al.[56] found that overall survival was affected by transfusions whereas cancer related death was not. This finding, which is confirmed by others,[57] suggests that there are other factors related to transfusions which are unfavorable for prognosis regarding other causes of death.

It had been suggested that the studies which did not find a significant effect of transfusions were too small in number of patients to show an effect, due to a too large probability of a type II error. Indeed, several of these studies were rather small but there were also studies including over 500 patients in which no effect of blood transfusions was found.[58,59]

Recently two meta-analyses on these clinical studies were published. The great advantage of a meta-analysis is that the sample size is increased considerably. In a meta-analysis which investigates the effect of blood transfusions on colorectal cancer, however, all the disadvantages related to observational studies still persist. Furthermore, only a few of the studies were prospective in design.[60,61] Therefore, a meta-analysis of all these studies cannot establish whether a negative association between blood transfusions and cancer prognosis is causal or not. Also, the phenomenon of publication bias (publication of positive outcomes might be more likely, leading to an underrepresentation of negative studies) is a potential problem in any meta-analysis.

In the study by Chung et al.[62] a total of 20 papers were reviewed, including over 5000 patients. The authors found that the cumulative odds-ratio of negative outcomes after perioperative blood transfusions was 1.80 for recurrence and 1.76 for death from cancer

(both $p<0.001$). The authors recognized that their results did not resolve the question of causality, but their analysis well defined the magnitude of the transfusion effect.
In the meta-analysis by Vamvakas and Moore[63] the data of 11 clinical studies were pooled and the studies in which possible confounders were not specified were left out from their analysis. Although the studies used in this analysis were the ones with good controlling for confounders, the study still showed a significant increase in the combined risk of cancer recurrence or cancer related death in the transfused patients of 37%. Yet the authors concluded that, considering the extent of residual confounding present in the pooled results, this entire average transfusion effect could be ascribed to the effect of uncontrolled confounding. Although these meta-analyses showed a significant negative relationship between blood transfusions and colorectal cancer prognosis, both studies are inconclusive with regard to the causality of this relationship.
Recently Houbiers et al.[64] presented a meta-analysis in which possible confounders are identified by analysing differences between subgroups of patients. It was suggested that more left sided tumors, more Dukes' C tumors, and older patients were present in the transfused group. These imbalances could explain the worse prognosis of transfused patients as compared with patients who did not receive transfusions.
After so many clinical reports investigating the association between blood transfusions and prognosis in colorectal cancer patients the exact solution for this clinical important problem is still not given.

Blood transfusions and postoperative infectious complications

Transfusion-induced immunosuppression might increase the susceptibility to bacterial infectious complications after surgical procedures. Tartter[65] was the first to demonstrate that in colorectal cancer patients who were transfused the rate of postoperative infectious complications was increased as compared with those patients who did not receive transfusions; infectious complications occurred in 25% and 4%, respectively. Multivariate analysis identified blood transfusion and hematocrit as significant independent risk factors for infections. Surprisingly, the patients with normal hematocrits who received transfusion had the highest rate of infections (33%). Wobbes et al.[66] found that the patients with multiple transfusions (over 4 units) had the highest rate of infections, and stated that the amount of transfusions was important for the increased susceptibility to infections. Transfusions also were a significant predictor of postoperative infections in patients with abdominal trauma or burn wounds[67,68] and transfusion even was a predictor of infections in patients transfused for gastrointestinal hemorrhage without surgery.[69] That the increased susceptibility to infections may be a result of transfusion-induced immunosupression has been supported by several animal studies. Waymack et al.[70,71] found that the function of macrophages was impaired by blood transfusion and that the

resistance to bacterial peritonitis was decreased after allogeneic blood transfusions. However, as for cancer recurrence the same limitations of these studies applied for infectious complications in relation to blood transfusions. Statistical analysis can evaluate different risk factors simultaneously and predict whether these factors have an independent effect. It is, however, impossible to exclude interactions between different variables. For example, the risk of infections is related to the extent of the operation, the amount of blood loss, and whether transfusion are given or not. These factors are that strong related to each other that it is difficult to point out which one is responsible for increased susceptibility to infections.

Alternatives for allogeneic blood transfusion

Being aware of the risks of allogeneic blood transfusions made clinicians try to reduce the exposure to allogeneic blood to a minimum. Naturally, blood transfusions should only be given when it is absolutely necessary. Tartter et al.[72] determined that 25% of the blood transfusions in colorectal cancer surgery were unnecessary and could be prevented by simply checking the hematocrit before transfusion. The most logical but not always possible solution will be the reduction of blood loss. Besides meticulous surgery and hemostatis serveral anesthesiologic techniques has been performed to reduce blood loss. For example, controlled hypotension, positioning methods, and regional anesthesia. Also drugs such as aprotinin, dipyridamole, and desmopressine have shown to have some value in reducing perioperative blood loss. However, for a large group of patients transfusions are still required and therefore it is worthwhile looking for the best alternative for allogeneic blood.

Autologous blood transfusions

Since autologous blood transfusions are, undoubtedly, the safest blood to transfuse several techniques have been performed to obtain autologous blood for transfusion.

Autologous blood transfusion can be performed in three ways:

1. Predeposit autologous blood donation, by which blood is collected by preoperative phlebotomies. This procedure has great popularity because of its safety. Although some studies doubted whether this is suitable in colorectal cancer patients,[73] it showed to reduce the exposure to allogeneic blood remarkably.[74,75] Predeposit autologous blood donation can be potentiated by the use of recombinant human erythropoietin,[76,77] which by itself also can reduce the need for transfusions perioperatively.[78,79]
2. Hemodilution, by which blood can be withdrawn immediately preoperatively or intraoperatively. The circulation will remain normovolemic due to infusion of colloid solutions. Hemodilution also can be performed hypervolemicly, by which no blood is withdrawn but as a result of colloid infusion the hematocrit is lowered and therefore the

loss of erythrocytes is reduced during surgery. This method has proven its value in Jehovah's Witnesses who refuse blood transfusions of any type.[80]
3. Intraoperative blood salvage from the operation field by means of a cell saver. The latter has proven to be a very useful way in reducing exposure to allogeneic blood especially in vascular and orthopedic surgery.[81] However, because of the fear of transfusing cancer cells and microorganisms into the patient's circulation the value of this technique in colorectal cancer surgery is questionable.

Aims of the studies

As discussed above the material present in the literature about the effect of blood transfusion-induced immunosuppression on the prognosis of cancer patients is still inconclusive. Several retrospective studies indeed found that blood transfusions are related to a poor prognosis in colorectal cancer patients. Experimental studies showed that growth of certain tumors was enhanced after allogeneic blood transfusions, which suggests that the relationship between blood transfusions and impaired cancer survival might be causal. However, there are also conflicting data from both experimental and clinical studies. Transfusions are given either because of the disease or as a result of surgical treatment. Therefore it is questionable, whenever a relationship between transfusions and cancer prognosis would exist, whether this relationship is causal or indirect. The need for transfusion could be an indicator of other prognostic factors, which are unknown, and therefore blood transfusion is coincidentally related to prognosis. The only way to resolve the question of causality is to perform a randomized trial comparing transfused patients with patients who did receive transfusions. Unfortunately, it is disputable whether such a trial design is ethically justified. Although the causality of the putative relationship can not be resolved completely it is possible to investigate whether the effects of allogeneic blood transfusions can be overcome. We have chosen predeposit autologous blood transfusion as the alternative for allogeneic blood transfusions in colorectal cancer surgery. This choice was based on the experimental findings that autologous blood transfusions are immunological neutral.[82,83] Therefore, the use of a predeposit autologous blood transfusion program could be an option to overcome the putative detrimental effects of allogeneic blood transfusions in colorectal cancer patients. The proper way to investigate the value of this method is a randomized trial.

The aims of the studies presented in this thesis are in the first place, to investigate whether the reduction of the exposure to allogeneic blood transfusions by means of a predeposit autologous blood donation program:

1. Can improve the survival in colorectal cancer patients (Chapter 2).
2. Can increase the disease-free survival in colorectal cancer patients (Chapter 2).
3. Can decrease the rate of postoperative infectious complications after colorectal cancer surgery (Chapter 5).

Secondly, it is tried to elucidate the putative detrimental relationship between:

1. Blood transfusions and colorectal cancer prognosis (Chapter 2, 3, and 4).
2. Blood transfusions and postoperative infectious complications after colorectal cancer surgery (Chapter 5).

Thirdly, to investigate whether autologous blood donation itself:

1. Can affect the immunological status of colorectal cancer patients (Chapter 6).
2. Can affect the prognosis and infectious complications after colorectal cancer surgery (Chapter 7).

References

1. Walker RH. Special report: Transfusion risks. Am J Clin Path 1987; 88:374-8.
2. Ho DD, Pomerantz RJ, Kaplan JC. Pathogenesis of infetion with human immunodeficiency virus. N Engl J Med 1987; 317:278-86.
3. Henle W, Henle G, Scriba M, et al. Antibody responses to the Epstein-Barr virus and cytomegalovirus after open heart and other surgery. N Engl J Med 1970; 282:1068-74.
4. Okochi K, Sato M, Himona Y. A retrospective study on transmission of adult T-cell leukemia virus by blood transfusion: seroconvesion in recipients. Vox Sang 1984; 465:245-53.
5. Cohen ND, Munoz A, Reitz BA, et al. Transmission of retroviruses by blood transfusion of screened blood in patients undergoing cardiac surgery. N Engl J Med 1989; 320:1172-5.
6. van der Poel CL, Reesink HW, Schaasberg W, et al. Infectivity of blood seropositive for hepatitis C virus antibodies. Lancet 1990; 335:558-60.
7. Opelz G, Sengar DPS, Mickey MR, Terasaki PI. Effect of blood transfusion on subsequent kidney transplants. Transplant Proc 1973; 5:253-9.
8. George CD, Morello PJ. Immunologic effects of blood transfusion upon renal transplantation, tumor operations, and bacterial infections. Am J Surg 1986; 152:329-37.
9. Fischer E, Lenhard V, Seifert P, Kluge A, Johannsen R. Blood transfusion-induced suppression of cellular immunity in man. Hum Immunol 1980; 3:187-93.
10. Klatzmann D, Bensussan A, Gluckman JC, et al. Blood transfusions suppress lymphocyte reactivity in uraemic patients. II. Evidence for soluble suppressor factors. Transplantation 1983; 36:337-40.
11. Watson MA, Briggs JD, Diamandopoulos AA, Hamilton DNH, Dick HM. Endogenous cell-mediated immunity, blood transfusion and outcome of renal transplantation. Lancet 1979; 2:1323-6.
12. Tartter PI, Heimann TM. Aufses AH. Blood transfusion, skin test reactivity, and lymphocytes in inflammatory bowel disease. Am J Surg 1986; 151:358-61
13. Waymack JP, Gallon L, Barcelli U, Trocki O, Alexander JW. Effect of blood transfusions on immune function. III. Alterations in macrophage arachidonic acid metabolism. Arch Surg 1987; 122:56-60.
14. Ford CD, Warnick CT, Sheets S, Quist R, Stevens LE. Blood trasnfusions lwer natural killer cell activity. Transplant Proc 1987; 19:1456-7.
15. Lenhard V, Gemsa D, Opelz G. Transfusion-induced release of prostaglandin E_2 and its role in the activation of T suppressor cells. Transplant Proc 1985; 17:2380-2.
16. Shelby J. The role of eicosanoids in the transfusion effect. Transplant Proc 1998; 20:1217-8.
17. Kaplan J, Sarnaik S, Gitlin J, Lusher J. Diminished helper/suppressor lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions. Blood 1984; 64:308-10.
18. IJzermans JNM. Immunoregulatory and antitumor effects of interferon-γ. Thesis 1991, Erasmus University Rotterdam, The Netherlands.
19. Brunson ME, Alexander JW. Mechanisms of transfusion-induced immunosuppression. Transfusion 1990; 30:651-8.
20. Blumberg N, Heal JM. Effects of transfusion on immune function, cancer recurrence and infection. Arch Pathol Lab Med 1994; 118:371-9.
21. Tartter PI, Francis DMA. Blood transfusion and tumor growth. Transplant Proc 1988; 20:1108-11.
22. van Twuyver E, Mooijaart RJD, ten Berge IJM, et al. Pretransplantation blood transfusion revisited. N Engl J Med 1991; 325:1210-3.
23. Gantt CL. Red blood cells for cancer patients. Lancet 1981; 2:363.
24. Rosenberg SA, Yang JC, Topalina SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high dose bolus interleukin-2. JAMA 1994; 271:907-13.
25. Moertel CG, Fleming TR, Macdonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990; 322:352-8.
26. Laurie JA, Moertel CG, Fleming TR, et al. Surgical adjuvant therapy of large-bowel carcinoma: an evaluation of levamisole and the combination of levamisole and fluorouracil. J Clin Oncol 1989; 7:1447-56.
27. Rosenberg SA, The development of new immunotherapies for the treatment of cancer with interleukin-2. Ann Surg 1988; 208:121-34

28. Riethmüller G, Schneider-Gädicke E, Schlimok G, et al. Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. Lancet 1994; 343:1177-83.
29. Francis DMA, Shenton BK. Blood transfusion and tumour growth, evidence from laboratory animals. Lancet 1981; 2:871.
30. Jeekel J, Eggermont A, Heystek GA, Marquet RL. Inhibition of tumour growth by blood transfusions in the rat. Eur Surg Res 1982; 14:114-5.
31. Oikawa T, Hosokawa M, Imamura M, et al. Anti-tumor immunity by normal allogeneic blood transfusions in the rat. Clin Exp Immunol 1977; 27: 549-54.
32. Singh SK. Modification of tumor growth by blood transfusion and perioperative procedures; a study in rats. Thesis 1988, Erasmus University Rotterdam, The Netherlands.
33. Marquet RL, de Bruin RWF, Dallinga R, Singh SK, Jeekel J. Modulation of tumor growth by allogeneic blood transfusion. J Cancer Res Clin Oncol 1986; 111:50-3.
34. Clarke PJ, Tarin D. Effect of pre-operative blood transfusion on tumour metastases. Br J Surg 1987; 74:520-4.
35. Zeller WJ, Scholler P, Robler W, et al. Allogeneic blood transfusions and experimental tumor growth. Transplant Proc 1986; 18:1448-9.
36. Blajchman MA, Bardossy L, Carmen R, et al. Allogeneic blood transfusion induced enhancement of tumor growth: two animal models showing amelioration by leukodepletion and passive transfer using spleen cells. Blood 1993; 81:1880-2.
37. Marquet RL, Busch ORC, Singh SK, Hoynck van Papendrecht, Jeekel J. The influence of anesthesia, surgery, blood loss and transfusions on immunity and tumor growth in an animal model. In: Houbiers JGA (ed.), Blood transfusion in cancer surgery, Boerhaave Committee Leiden 1993; 49-62.
38. Singh SK, Marquet RL, Westbroek DL, Jeekel J. Enhanced tumor growth of artificial tumor metastases following blood transfusion: the effect of erythrocytes, leukocytes, and plasma transfusion. Eur J Cancer Clin Oncol 1987; 23:1537-40.
39. Singh SK, Marquet RL, de Bruin RW, Hop WC, Westbroek DL, Jeekel J. Consequences of blood loss on growth of artificial metastases. Br J Surg 1988; 75:377-9.
40. Hoynck van Papendrecht MAW, Busch ORC, Jeekel J, Marquet RL. The influence of blood loss on tumour growth: effect and mechanism in an experimental model. Neth J Surg 1991; 43:85-8.
41. Burrows L, Tartter P. Effect of blood transfusions on colonic malignancy recurrence rate. Lancet 1982; 2:662.
42. Tartter PI. Perioperative blood transfusion and colorectal cancer recurrence: a review. J Surg Oncol 1988; 39:197-200.
43. Wu HS, Little AG. Perioperative blood transfusions and cancer recurrence. J Clin Oncol 1988; 6:1348-54.
44. Schriemer PA, Longnecker DE, Mintz PD. The possible immunosuppressive effects of perioperative blood transfusion in cancer patients. Anesthesiology 1988; 68:422-8.
45. Blumberg N, Heal JM. Transfusion and host defenses against cancer recurrence and infection. Transfusion 1989; 29:236-45.
46. Francis DMA. Relationship between blood transfusion and tumour behaviour. Br J Surg 1991; 78:1420-8.
47. Houbiers JGA. Blood transfusion, immune response and colorectal cancer. Thesis 1994, University of Leiden, The Netherlands.
48. Blair SD, Janvrin SB. Relation between cancer of the colon and blood transfusion. Br Med J 1985; 290:1516-7.
49. Bentzen SM, Balslev I, Pedersen M, et al. Blood transfusion and prognosis in Dukes' B and C colorectal cancer. Eur J Cancer 1990; 26:457-63.
50. Nathanson SD, Tilley BC, Schultz L, Smith RF. Perioperative allogeneic blood transfusions; Survival in patients with resected carcinomas of the colon and rectum. Arch Surg 1985; 120:734-8.
51. Hodgson WJB, Lowenfels AB. Blood transfusions and recurrence rates in colonic malignancy. Lancet 1982; 2:1047.
52. Parrott NR, Lennard TW, Taylor RM, et al. Effect of perioperative blood transfusion on recurrence of colorectal cancer. Br J Surg 1986; 73:970-3.

53. van Lawick van Pabst WP, Langenhorst BLAM, Mulder PGH, Marquet RL, Jeekel J. Effect of perioperative blood loss and perioperative blood transfusion on colorectal cancer survival. Eur J Cancer Clin Oncol 1988; 24:741-7.
54. Agarwal M, Blumberg N. Colon cancer patients transfused perioperatively have an increased incidence of recurrence. Transfusion 1983; 23:421.
55. Foster RS, Costanza MC, Foster JC, Wanner MC, Foster CB. Adverse relationship between blood transfusions and survival after colectomy for colon cancer. Cancer 1985; 55:1195-201.
56. Voogt PJ, van de Velde CJH, Brand A, et al. Perioperative blood transfusion and cancer prognosis; different effects of blood transfusion on prognosis of colon and breast cancer patients. Cancer 1987; 59:836-43.
57. Liewald F, Wirsching RP, Zülke C, Demmel N, Mempel W. Influence of blood transfusions on tumor recurrence and survival rate in colorectal carcinoma. Eur J Cancer 1990; 26:327-35.
58. Crowson MC, Hallissey MT, Kiff RS, Keingston RD, Fielding JWL. Blood transfusion in colorectal cancer. Br J Surg 1989; 76:522-3.
59. Cheslyn-Curtis S, Fielding LP, Hittinger R, Fry JS, Phillips RKS. Large bowel cancer: the effect of perioperative blood transfusion on outcome. Ann R Coll Surg Engl 1990; 72:53-9.
60. Frankish PD, McNee RK, Alley PG, Woodfield DG. Relation between cancer of the colon and blood transfusion. Br Med J 1985; 290:1827.
61. Tartter PI. The association of perioperative blood transfusion with colorectal cancer recurrence. Ann Surg 1992; 216:633-8.
62. Chung M, Steinmetz OK, Gordon PH. Perioperative blood transfusion and outcome after resection for colorectal carcinoma. Br J Surg 1993; 80:427-32.
63. Vamvakas E, Moore SB. Perioperative blood transfusion and colorectal cancer recurrence: a qualitative statistical overview and meta-analysis. Transfusion 1993; 33:754-65.
64. Houbiers JGA, Brand A, Speirings EHT, van de Velde CJH, van Houwelingen JC. Meta-analysis identified confounders responsible for the association between blood transfusion and colorectal cancer recurrence. In: Houbiers JGA. Blood transfusion, immune response and colorectal cancer. Thesis 1994, University of Leiden, The Netherlands :89-102.
65. Tartter PI. Blood transfusion and infectious complications following colorectal cancer surgery. Br J Surg 1988; 75:789-92.
66. Wobbes T, Bemelmans BLH, Kuypers JHC, Beerthuizen GIJM, Theeuwes AGM. Risk of postoperative septic complications after abdominal surgical treatment in relation to perioperative blood transfusion. Surg Gynecol Obstet 1990; 171:59-62.
67. Nichols RL, Smith JW, Klein DB, et al. Risk of infection after penetrating abdominal trauma. N Engl J Med 1984; 311:1065-70.
68. Polk HC, George CD, Willhausen SR, et al. A systematic study of host defense processes in badly injured patients. Ann Surg 1986; 204:282-99.
69. Christou NV, Meakins JL, Gotto D, MacLean LD. Influence of gastrointestinal bleeding on host defense and susceptibility to infection. Surg Forum 1979; 30:46-7.
70. Waymack JP, Rapien J, Garnett D, Tweddell JS, Alexander JW. Effect of transfusion on immune function in a traumatized animal model. Arch Surg 1986; 121:50-5.
71. Waymack JP, Warden GD, Alexander JW, Miskell P, Gonce S. Effect of blood transfusion and anesthesia on resistance to bacterial peritonitis. J Surg Res 1987; 42:528-35.
72. Tartter PI, Barron DM. Unnecessary blood transfusion in elective colorectal cancer surgery. Transfusion 1985; 25:113-5.
73. Harrison S, Steele RJC, Johnston AK, Jones JA, Morris DL, Hardcastle JD. Predeposit autologous blood transfusion in patients with colorectal cancer: a feasibility study. Br J Surg 1992; 79:355-7.
74. Hoynck van Papendrecht MAW, Hop W, Langenhorst BLAM, Kothe FC, Marquet RL, Jeekel J. Feasibility of a predeposit autologous blood donation program in colorectal cancer patients: Results from a randomized clinical study. Vox Sang 1992; 62:102-7.
75. Hoynck van Papendrecht MAWMC. Prevention of blood transfusions in oncologic surgery. Thesis 1991, Erasmus University Rotterdam, The Netherlands.
76. Biesma DH. Autologous blood transfusion, erythropoietin and iron metabolism. Thesis 1994, University of Utrecht, The Netherlands.
77. Goodnough LT, Rudnick S, Price TH, et al. Increased preoperative collection of autologous blood with recombinant human erythropoietin therapy. N Engl J Med 1989; 321:1163-8.

78. Rothstein P, Roye D, Verdisco L, Stern L. Preoperative use of erythropoietin in an adolescent Jehovah's Witness. Anesthesiology 1990; 73:568-70.
79. Busch ORC, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Experimental and clinical results of perioperative treatment with rHuEPO. In: Pagel H, Weiss C, Jelkmann W (eds). Pathophysiology and pharmacology of erythropoietin. Springer-Verlag 1992; 315-20.
80. Trouwborst A, van Woerkens ECSM, van Daele M, Tenbrink R. Acute hypervolaemic haemodilution to avoid blood transfusion during major surgery. Lancet 1990; 336:1295-7.
81. Koopman-van Gemert AWMM. Perioperative autotransfusion by means of a blood cell separator. Thesis 1993, University of Nijmegen, The Netherlands.
82. Singh SK, Marquet RL, de Bruin RWF, Westbroek DL, Jeekel J. Promotion of tumor growth by blood transfusions. Transplant Proc 1987; 19:1473-4.
83. Waymack JP, Chance WT. Effect of blood transfusions on immune function: IV. Effect on tumor growth. J Surg Oncol 1988; 39:159-64.

CHAPTER 2

BLOOD TRANSFUSIONS AND PROGNOSIS IN COLORECTAL CANCER

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Published in:
New England Journal of Medicine 1993; 328: 1372-1376.

Chapter 2

Summary

Blood transfusions may adversely affect the prognosis of patients treated surgically for cancer, although definite proof of this adverse effect has not been reported.

We carried out a randomized trial to investigate whether the prognosis in patients with colorectal cancer would be improved by a program of autologous blood transfusion as compared with the current practice of allogeneic transfusion. Patients in the autologous group were required to donate two units of blood before surgery.

A total of 475 patients were evaluated. We found no significant difference in prognosis between the allogeneic group (N=236) and the autologous group (N=239); colorectal cancer-specific survival rates at 4 years were 67% and 62%, respectively (p=0.39). Among the 423 patients who underwent curative surgery, 66% of those in the allogeneic group and 63% of those in the autologous group had no recurrence of colorectal cancer at 4 years (p=0.93).

We also found that the risk of recurrence was significantly increased in patients who received blood transfusions, either allogeneic or autologous, as compared with patients who did not require transfusions; the relative recurrence rates were 2.1 (p=0.01) and 1.8 (p=0.04), respectively; these rates did not differ significantly from each other.

The use of autologous blood as compared with allogeneic blood for transfusion does not improve the prognosis in patients with colorectal cancer. Regardless of their type, transfusions are associated with poor prognosis, probably because of the circumstances that necessitate them.

Introduction

Perioperative blood transfusions may have a deleterious effect on the survival of patients with a variety of solid tumors,[1,2] possibly because of an immunosuppressive effect.[3,4] This possibility is supported by studies in animals in which tumor growth was enhanced after allogeneic transfusion,[5,6] although conflicting results have also been reported.[7,8] A poor prognosis after blood transfusions has been noted especially in patients with colorectal cancer.

In the studies of the effect of blood transfusions in patients with cancer, the patients were given the transfusions either because of their disease or because transfusion was necessary during surgical treatment. Therefore, the question remains whether the relation between blood transfusion and poor prognosis is causal or coincidental.[9] The need for transfusion could be an indicator of other prognostic factors that are either unknown or difficult to quantify, such as the extent of the tumor and the dissection, the skill of the surgeon, and the nutritional state of the patient.

A randomized trial is the only way in which possible bias in the selection of patients can be avoided. Randomization between transfusion and no transfusion is impossible, however, because giving patients transfusions when there is no medical indication and withholding transfusions that are indicated are ethically unacceptable. Since autologous blood is the safest blood to use in transfusion, comparing the effects of allogeneic and autologous blood transfusions would be a logical option.

We therefore conducted a randomized multicenter trial in patients with colorectal cancer to determine whether autologous blood transfusion would reduce the rate of recurrence of cancer and improve the survival as compared with allogeneic transfusion. We previously reported that a notable reduction in the number of allogeneic transfusions can be achieved with a program of autologous blood transfusion.[10]

Methods

The study was conducted in 14 hospitals in The Netherlands and 1 hospital in England and was approved by the ethics committees of all the participating hospitals. After written informed consent had been obtained, eligible patients were randomly assigned to either the allogeneic group or the autologous group, with stratification according to participating hospital. Patients were enrolled from August 1986 to November 1991, when the planned enrollment was reached.

Eligibility of patients

Patients scheduled for a potentially curative resection of cancer of the colon or rectum were eligible for enrollment if they fulfilled the criteria of the American Association of

Blood Banks for autologous blood donation in anticipation of surgery.[11] These criteria required the absence of severe cardiovascular or respiratory disease, no history of epilepsy after infancy, and a hemoglobin concentration above 11.3 g/dl (7 mmol/l). In addition, patients had to have no evidence of metastatic disease, on the basis of chest radiography and ultrasonography of the liver; no other cancer except basal-cell carcinoma of the skin or in situ carcinoma of the cervix; no evidence of ulcerative colitis, familial polyposis, or a fixed rectal carcinoma requiring preoperative radiation therapy; and no history of blood transfusion during the three months before randomization. No adjuvant therapy was allowed except irradiation. If metastatic or recurrent disease developed in a patient during follow-up, all available therapies were allowed.

Procedures for donation and transfusion

Patients randomly assigned to the autologous group were required to donate blood twice. The minimal interval between the two donations was 72 hours, and the second donation had to occur not later than five days before surgery. At each donation, 450 ml of blood was obtained by standard procedures. The patients were treated with oral iron supplementation immediately after randomization.

The collected blood was separated into packed red cells and fresh-frozen plasma, except at one hospital, where autologous blood was given in transfusion as whole blood. The packed red cells and whole blood were stored at 4°C. Allogeneic blood was always given in transfusion as packed red cells. Standard rules for transfusion were used for both groups. Packed red cells could be given only if the loss of blood exceeded 500 ml or if the hemoglobin concentration dropped below 10.5 g/dl (6.5 mmol/l). If this hemoglobin concentration was not achieved after two autologous transfusions, additional allogeneic transfusions were made. In both groups fresh-frozen plasma was given when indicated.

Surgery and histopathological assessment

Standard surgical procedures were used. The operative specimens were classified according to Dukes' classification as modified by Turnbull.[12] A tumor confined to the bowel wall was classified as Dukes' A; a tumor extending through the serosa into the pericolic fat as Dukes' B; the presence of regional lymph nodes containing metastases as Dukes' C; and the presence of distant metastases or unresectable tumor as Dukes' D. All patients who had residual tumor evident only on microscopical examination received postoperative radiotherapy. These patients and those who had en bloc resection of adjacent organs were not considered as having Dukes' D, but rather as having Dukes' B or C disease.

Follow-up and criteria for recurrent cancer

The patients were evaluated every three months during the first two years after surgery and every six months thereafter. Each evaluation consisted of a history, a physical

examination, and blood tests (to measure the concentrations of hemoglobin and serum carcino-embryonic antigen). Ultrasonography of the liver was performed every six months for three years and each year thereafter. Chest films and colonoscopy were done yearly.
Characteristic abnormalities detected on physical examination or on chest radiography, liver ultrasonography, or abdominal computed tomography were accepted as evidence of metastatic or recurrent disease. If possible, the presence of metastatic or recurrent disease was confirmed by histologic or cytologic examination. Increased serum concentrations of carcino-embryonic antigen without evidence of recurrence at suspected anatomical sites were not considered to indicate metastatic or recurrent disease.

Statistical analysis
Categorical data were compared by the chi-square test, and continuous data by the Mann-Whitney test. The major end points were disease-free survival and colorectal cancer-specific survival (including as events all patients who died of colorectal cancer, without regard to other causes of death), both as determined from the time of surgery and calculated according to the Kaplan-Meier method. The log-rank test was used to compare these end points. Multivariate analysis was performed by proportional-hazards analysis[13] to obtain a higher level of precision in the comparison of the randomized groups. Two-sided p values of 0.05 or less were considered statistically significant. P values calculated with adjustment for Dukes' stage are indicated as adjusted p values in the text.
Patients in whom a secondary primary tumor developed outside the colon were withdrawn from study with regard to the calculation of disease-free survival. Metachronous tumors in the colon, however, were defined as representing recurrent disease. Postoperative deaths, defined as deaths occurring within 30 days after surgery, and deaths occurring more than 30 days after surgery due to postoperative complications were counted as deaths due to cancer.
Because the analysis of overall survival and colorectal cancer-specific survival gave similar results, only the results of colorectal cancer-specific survival are reported.
Except for the patients who did not have colorectal cancer at the time of surgery, all randomized patients were primarily evaluated according to the intention-to-treat principle. In addition, exploratory analyses were performed according to the number and type of transfusions received.

Results

Characteristics of the patients
A total of 510 patients were enrolled in the study. Thirty-five patients (7%) were excluded because they did not have colorectal cancer at the time of surgery. The characteristics of the remaining 475 patients are shown in Table 2.1.

Table 2.1. Characteristics of the patients with colorectal cancer in the allogeneic and autologous groups.

	Allogeneic group (N = 236)		Autologous group (N = 239)	
Median age (years, range)	68	(33-89)	66	(31-88)
Sex				
Male	132	(56%)	141	(59%)
Female	104	(44%)	98	(41%)
Tumor location				
Ascending colon	24	(10%)	16	(7%)
Flexures and transverse colon	12	(5%)	16	(7%)
Descending colon and sigmoid	62	(26%)	65	(27%)
Rectosigmoid and rectum	132	(56%)	135	(56%)
Multiple primary tumors	6	(3%)	7	(3%)
Dukes' classification*				
A	53	(23%)	55	(23%)
B	85	(36%)	80	(34%)
C	78	(33%)	72	(31%)
D	18	(8%)	29	(12%)
Histologic differentiation				
Well	35	(15%)	33	(14%)
Moderate	169	(72%)	172	(72%)
Poor	29	(12%)	29	(12%)
Unknown	3	(1%)	5	(2%)
Adjacent organ fixation**	15	(7%)	17	(8%)
Adjuvant irradiation	15	(7%)	16	(8%)

* Does not include patients who did not undergo surgery (two in the allogeneic group and three in the autologous group).

** Does not include patients with Dukes' D stage.

None of the characteristics differed significantly between the two groups. Twenty-six of the 239 patients in the autologous group (11%) did not donate blood; the majority of these were refused by the blood bank because they did not fulfill the criteria of the American Association of Blood Banks. These patients were included in all analyses according to the intention-to-treat principle, as were all patients with metastatic disease or unresectable tumors. The median follow-up period was 2.5 years (range, 1 to 59 months), and no patient was lost to follow-up.

The perioperative hematologic values and use of transfusions are shown in Table 2.2. The number of patients in the autologous group who received allogeneic blood was half the number in the allogeneic group (28% vs. 56%, respectively; $p<0.001$).

Table 2.2. Hemoglobin concentrations, blood loss, and transfusions in patients with colorectal cancer in the allogeneic and autologous groups.

	Allogeneic group		Autologous group		p value
Hemoglobin concentration (g/dl)					
Base-line	14.5	(10.5-18.0)	14.4	(10.7-18.5)	n.s.
Immediately preoperative	14.1	(9.5-18.0)	12.5	(8.4-16.4)	<0.001
Discharge	12.5	(9.2-17.2)	12.2	(9.2-16.2)	n.s.
Blood loss (ml)	775	(100-11,500)	750	(100-6,500)	n.s.
Transfusions					
None	103	(44%)	61	(26%)	<0.001
Only autologous	-	-	112	(47%)	-
Allogeneic	133	(56%)	66	(28%)	<0.001

Hemoglobin concentrations and blood loss values are shown as medians with ranges in parentheses, and transfusions are shown as numbers of patients with percentages in parentheses.

Morbidity and mortality

Five eligible patients (two in the allogeneic group and three in the autologous group) did not have surgery. Four had more advanced disease than expected, and one patient died before surgery. Eight patients (three in the allogeneic group and five in the autologous group) died of postoperative complications. Postoperative infectious complications occurred in 26% of the patients (25% in the allogeneic group and 27% in the autologous group). There were no statistically significant differences between the two groups with respect to postoperative mortality and infectious complications.

Disease-free survival

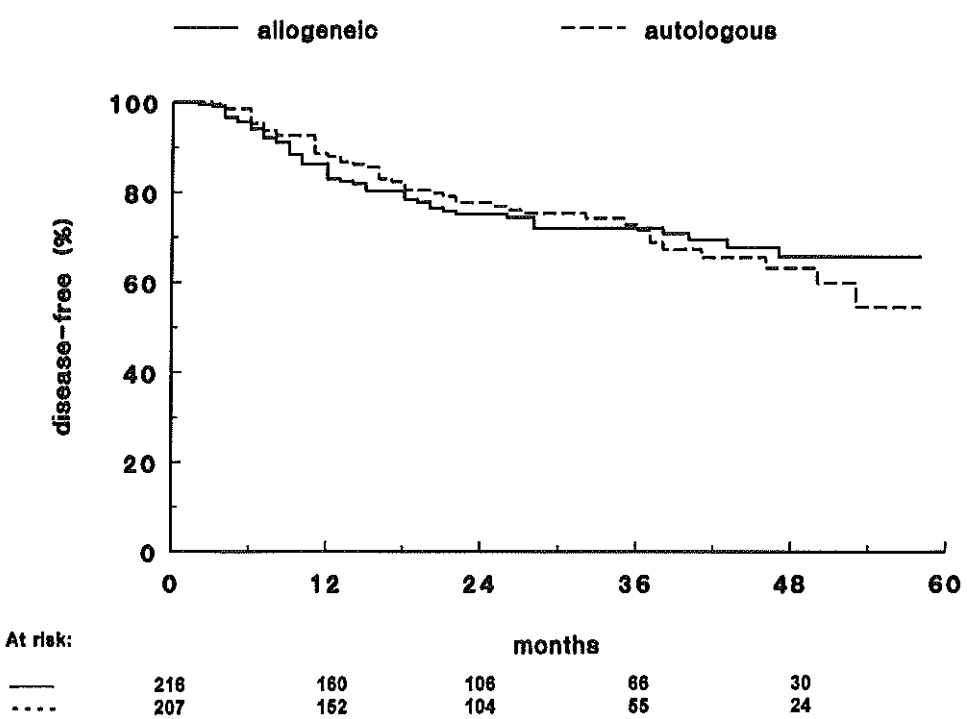
Of the 423 patients who underwent curative surgery (216 in the allogeneic group and 207 in the autologous group), 105 (54 in the allogeneic group and 51 in the autologous group) had recurrent disease (including three metachronous tumors). The disease-free survival of these 423 patients is shown in Figure 2.1. The plots for the two groups were almost identical (p=0.93). Also, there were no differences with regard to disease-free survival in each of the Dukes' stages. After adjusting for various factors, the multivariate analysis also revealed no difference between the groups in disease-free survival (Table 2.3). Six patients (two in the allogeneic group and four in the autologous group) had second primary tumors outside the colon during follow-up.

To explore the relation between blood transfusions and disease-free survival, we grouped the patients who underwent curative surgery according to the number and type of transfusions they received. The disease-free survival in the 143 patients who received no transfusions was significantly better (adjusted p=0.001) than that in the 280 who did

receive transfusions; at 4 years, it was 73% and 59%, respectively. Among the 280 patients who had transfusions, 136 received only allogeneic transfusions, 102 only autologous transfusions, and 42 transfusions of both types. The disease-free survival at 4 years in these three groups was 56%, 62%, and 66%, respectively (adjusted p=0.50). No significant difference was found between the 49 patients who received no transfusions in the autologous group and the 94 such patients in the allogeneic group; the disease-free survival in these patients at 4 years was 69% and 75%, respectively.

Figure 2.1. Disease-free survival of all 423 colorectal cancer patients who underwent curative surgery comparing randomization.

The disease-free survival at 4 years is 66% in the allogeneic group and 63% in the autologous group (p=0.93). The 95% confidence interval for the difference (autologous minus allogeneic) between these percentages ranged from -16% to +10%.



Because the number of autologous transfusions was limited to two, further comparisons were made between the 75 patients in the allogeneic group who received one or two allogeneic transfusions and the 102 patients in the autologous group who received one or two autologous transfusions but no allogeneic transfusions. The disease-free survival was significantly worse in the patients in both groups who received transfusions than in the 143 patients who did not, whereas the disease-free survival of the patients who received transfusions in both groups did not differ significantly from each other (Table 2.4).
Analysis of the results with respect to the use of fresh-frozen plasma revealed no relation between its use and disease-free survival. This was true whether the patients received blood transfusions or not.

Table 2.3. Multivariate analysis of factors with respect to disease-free survival in 423 patients with colorectal cancer who underwent curative surgery.*

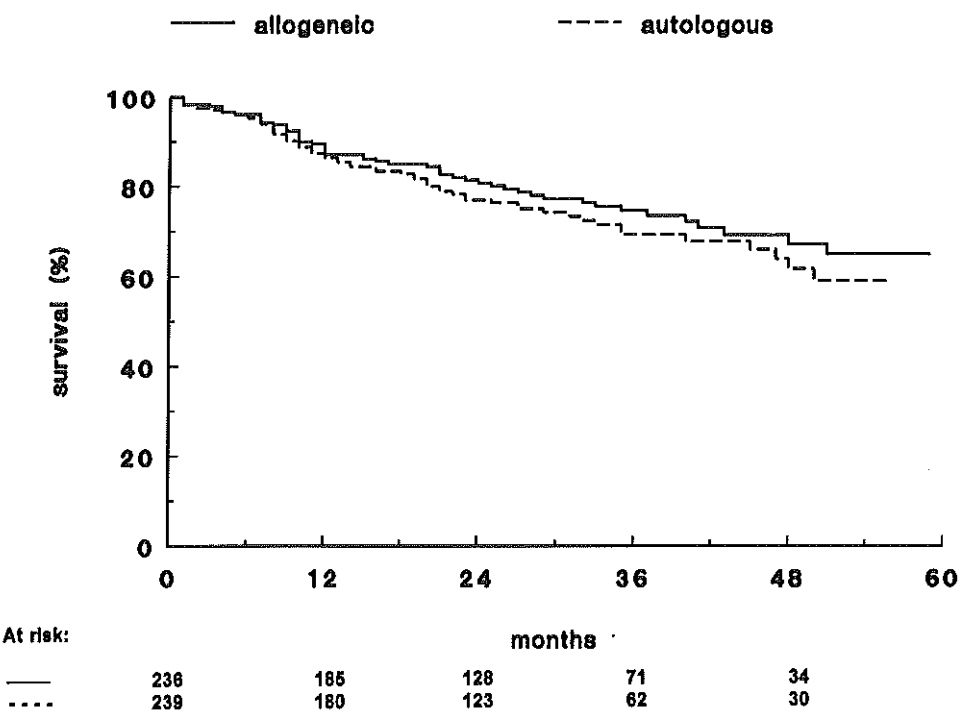
Factor	Relative recurrence rate	95% confidence interval	p value
Randomization			
Allogeneic group	1	-	-
Autologous group	1.1	0.7 - 1.6	0.74
Dukes' classification			
A	1	-	-
B	4.0	1.7 - 9.5	0.002
C	10.8	4.7 - 25.1	<0.001

* Other factors investigated (age and sex of patients, tumor location, adjacent organ fixation, degree of differentiation and size of the tumor) were of no significant additional predictive value with respect to disease-free survival, and the effect of randomization was not significantly influenced by any of these factors.

Colorectal cancer-specific survival
During the study, 114 patients died of colorectal cancer (53 in the allogeneic group and 61 in the autologous group). The survival of all 475 eligible patients in the two groups was similar (p=0.39) (Figure 2.2).
In addition to the Dukes' stage, the patient's age was also significantly related to colorectal cancer-specific survival (i.e., older patients generally did worse than younger ones). The ratio of the death rate in the autologous group to the death rate in the allogeneic group, after adjustment for Dukes' stage and age, was 1.1 (95% confidence interval, 0.8 to 1.7; p=0.66).

Figure 2.2. Colorectal cancer-specific survival of all 475 colorectal cancer patients comparing randomization.

The survival at 4 years is 67% in the allogeneic group and 62% in the autologous group (p=0.39). The 95% confidence interval for the difference (autologous minus allogeneic) between these percentages ranged from -18% to +7%.



As was the case for disease-free survival, the colorectal cancer-specific survival in the 423 patients who underwent curative surgery was significantly better (adjusted p<0.001) in the patients who did not receive transfusion than in those who did; the survival at 4 years was 88% and 65%, respectively. When the patients who received transfusions were subdivided according to the type of transfusions they received (allogeneic, autologous, or both), there were no significant differences (adjusted p=0.60) between the three subgroups; the survival at 4 years was 64%, 68%, and 63%, respectively. The survival of patients who did not receive transfusions did not differ significantly (adjusted p=0.86) between the two randomized groups; the survival of these patients at 4 years was 87% in the allogeneic group and 88% in the autologous group.

When the analysis was restricted to patients who had one or two transfusions of the same type, the patients receiving autologous transfusions and those receiving allogeneic transfusions both had worse survival rates than the patients without transfusions (Table 2.4).

No relation was found between survival and the transfusion of fresh-frozen plasma.

Table 2.4. Disease-free survival and colorectal cancer-specific survival according to transfusion status and Dukes' classification, in patients with colorectal cancer who underwent curative surgery.

Factor	Number of patients	Disease-free survival at 4 years	Relative recurrence rate*	Survival at 4 years	Relative death rate*
Transfusions					
None	143	73%	1	88%	1
1 or 2 allogeneic	75	59%	2.1†	67%	3.6†
1 or 2 autologous	102	62%	1.8†§	68%	2.8†§
Dukes' classification					
A	78	93%	1	94%	1
B	130	73%	4.3†	85%	1.2
C	112	39%	15.5†	50%	10.8†

* Relative rates of recurrence and death obtained by multivariate analysis.
† Significantly ($p<0.05$) different from no transfusions.
§ Not significantly different ($p>0.60$) from the group receiving 1 or 2 allogeneic transfusions.

Discussion

The results of the retrospective studies of the influence of blood transfusions on the survival and the recurrence rate in patients with colorectal cancer are conflicting. The studies in which the prognosis in patients receiving transfusions was poorer may have been biased by the selection of patients.[14,15] One way to avoid such confounding by indication[16] is to conduct a randomized trial comparing the effects of autologous and allogeneic blood transfusions.[17,18] The results of this study indicate that as compared with the use of allogeneic blood, the use of autologous blood either to avoid or to reduce exposure to allogeneic blood neither lowered the recurrence rate nor improved survival in patients who had undergone surgery for colorectal cancer. In accordance with some retrospective studies, the recurrence rate was higher in patients who had received transfusions than in those who had not. For patients given transfusions with allogeneic blood, the increase in the recurrence rate was similar to that in the patients who received only autologous blood. The same applied to the survival of the patients.

Recently, Ness et al.[19] reported no difference in survival in a nonrandomized study in which the effects of allogeneic and autologous blood were compared in patients undergoing radical surgery for prostate cancer. The results of a randomized study comparing both types of transfusion in patients with colorectal cancer was also presented recently.[20] In that study, which included only 120 patients, there were fewer recurrences in the autologous group, but on the basis of life-table analysis there were no statistically significant differences between the randomized groups.

An explanation for our findings could be that autologous blood induces the same adverse reactions as allogeneic blood. In animals in which allogeneic transfusions had an adverse effect, no such effect was described for syngeneic blood transfusions.[21,22] Since autologous blood transfusions in humans are comparable to syngeneic transfusions in animals, there is no experimental support for an effect of autologous transfusions on tumor growth. On the other hand, autologous transfusion requires the donation of blood. We have found in rats that the donation of blood can decrease natural killer cell activity and stimulate tumor growth.[23,24,25] Therefore, the patients in the autologous group could have had a lower natural killer cell activity than the patients in the allogeneic group at the time of surgery. However, among the patients who did not receive transfusions, there was no difference in survival between the patients in the autologous group, who donated blood, and those in the allogeneic group.

The most likely explanation for our findings is that there is no causal relation between blood transfusions and prognosis in patients with colorectal cancer. Thus, the findings in the retrospective studies in which blood transfusion was a determinant of prognosis were probably due to patient selection. We think it is not the blood transfusions themselves, but rather the circumstances necessitating the transfusions, that are the real determinant of prognosis. The need to give patients transfusions during the perioperative period is obviously determined by a number of factors, such as blood loss, the extent of the tumor and the dissection, and the skill of the surgeon, although we found tumor size not to be a determinant of prognosis in multivariate analysis. In some retrospective studies the groups receiving transfusions contained more patients with rectal tumors, who have a poorer prognosis than patients with colon cancer, than did the groups not receiving transfusions.[26,27] We found that the higher recurrence rates in both groups receiving transfusions, as compared with the rate in the group receiving no transfusions, were not affected by the location of the tumor. Because of our rules regarding transfusion, there was such a strong relation between blood loss and transfusion that it was impossible to separate these two factors. Other possible reasons for transfusion, such as the extent of the dissection and the skill of the surgeon are difficult to assess. Although it seems beneficial to operate on patients with colorectal cancer in such a way that blood transfusions are either avoided or minimized, there is no reason, with respect to either cancer recurrence or survival, to use a program of transfusion with autologous blood in patients undergoing surgery for colorectal cancer.

References

1. Blumberg N, Heal JM. Transfusion and host defenses against cancer recurrence and infection. Transfusion 1989; 29:236-45.
2. Francis DMA. Relationship between blood transfusion and tumour behaviour. Br J Surg 1991; 78:1420-8.
3. Gantt CL. Red blood cells for cancer patients. Lancet 1981; 2:363.
4. Burrows L, Tartter P. Effect of blood transfusions on colonic malignancy recurrence rate. Lancet 1982; 2:662.
5. Singh SK, Marquet RL, Westbroek DL, Jeekel J. Enhanced growth of artificial tumor metastases following blood transfusion: The effect of erythrocytes, leukocytes, and plasma transfusion. Eur J Cancer Clin Oncol 1987; 23:1537-40.
6. Francis DMA, Burren CP, Clunie GJA. Acceleration of B16 melanoma growth in mice after blood transfusion. Surgery 1987; 102:485-92.
7. Tartter PI, Francis DMA. Blood transfusion and tumor growth. Transplant Proc 1988; 20:1108-11.
8. Jeekel J, Eggermont AMM, Heystek GA, Marquet RL. Inhibition of tumor growth by blood transfusions in the rat. Eur Surg Res 1982; 14:124-5.
9. Brunson ME, Alexander JW. Mechanisms of transfusion-induced immunosuppression. Transfusion 1990; 30:651-8.
10. Hoynck van Papendrecht MAW, Hop W, Langenhorst BLAM, Kothe FC, Marquet RL, Jeekel J. Feasibility of a predeposit autologous blood donation program in colorectal cancer patients: Results from a randomized clinical study. Vox Sang 1992; 62:102-7.
11. Council on Scientific Affairs. Autologous blood transfusions. JAMA 1986; 256:2378-80.
12. Turnbull RB, Kyle K, Watson FR, Spratt J. Cancer of the colon: The influence of the no-touch isolation technic on survival rates. Ann Surg 1967; 166:420-5.
13. Cox DR. Regression models and life tables. J R Stat Soc [B] 1972; 34:187-202.
14. Bentzen SM, Balslev I, Pedersen M, et al. Blood transfusion and prognosis in Dukes' B and C colorectal cancer. Eur J Cancer 1990; 26:457-63.
15. Nathanson SD, Tilley BC, Schultz L, Smith RF. Perioperative allogeneic blood transfusions; Survival in patients with resected carcinomas of the colon and rectum. Arch Surg 1985; 120:734-8.
16. Miettinen OS. The need for randomization in the study of intended effects. Stat Med 1983; 2:267-71.
17. van Lawick van Pabst WP, Langenhorst BLAM, Mulder PGH, Marquet RL, Jeekel J. Effect of perioperative blood loss and perioperative blood transfusion on colorectal cancer survival. Eur J Cancer Clin Oncol 1988; 24:741-7.
18. Taylor RMR, Parrott NR. Red alert. Br J Surg 1988; 75:1049-50.
19. Ness PM, Walsh PC, Zahurak M, Baldwin ML, Piantadosi S. Prostate cancer recurrence in radical surgery patients receiving autologous or homologous blood. Transfusion 1992; 32:31-6.
20. Heiss MM, Jauch KW, Delanoff C, Mempel W, Schildberg FW. Blood transfusion modulated tumor recurrence; a randomized study of autologous versus homologous blood transfusion in colorectal cancer. Proc Am Soc Clin Oncol 1992; 11:172. abstract.
21. Singh SK, Marquet RL, de Bruin RWF, Westbroek DL, Jeekel J. Promotion of tumor growth by blood transfusions. Transplant Proc 1987; 19:1473-4.
22. Waymack JP, Chance WT. Effect of blood transfusions on immune function: IV. Effect on tumor growth. J Surg Oncol 1988; 39:159-64.
23. Singh SK, Marquet RL, de Bruin RWF, Hop WCJ, Westbroek DL, Jeekel J. Consequences of blood loss on growth of artificial metastases. Br J Surg 1988; 75:377-9.
24. Hoynck van Papendrecht MAW, Busch ORC, Jeekel J, Marquet RL. The influence of blood loss on tumour growth: effect and mechanism in an experimental model. Neth J Surg 1991; 43:85-8.
25. Herberman RB, Ortaldo JR. Natural killer cells: their role in defenses against disease. Science 1981; 214:24-30.
26. Weiden PL, Bean MA, Schultz P. Perioperative blood transfusion does not increase the risk of colorectal cancer recurrence. Cancer 1987; 60:870-4.
27. Frankish PD, McNee RK, Alley PG, Woodfield DG. Relation between cancer of the colon and blood transfusion. Br Med J 1985; 290:1827.

LETTERS TO THE EDITOR

BLOOD TRANSFUSIONS AND PROGNOSIS IN COLORECTAL CANCER

Published in:
New England Journal of Medicine 1993; 329: 1354-1356.

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To the Editor:

Busch et al. (May 13 issue)[1] fail to consider the possibility that both autologous and allogeneic blood may contain an immunosuppressive factor that could influence the rate of recurrence of colorectal cancer. Until recently, it was generally thought that the cellular components of transfused blood were responsible for the immunomodulatory effects of transfusion, particularly in renal transplantation. Evidence is accumulating, however, to implicate other components. In colorectal cancer, transfusions of plasma protein fractions and fresh-frozen plasma have been associated with increased tumor recurrence.[2,3] Experimental work suggests that humoral rather than cellular components may be more important in transfusion-associated immunosuppression, although this has not yet been shown in tumors in animals. In rats that undergo transfusion, the effects of pretransfusion storage of blood on the synthesis of prostaglandin E_2 by macrophages were greater than the effects due to genetic differences between blood donor and recipient.[4] Serum had the most potent effect, indicating that a major factor activating prostaglandin E_2-mediated immunosuppression in patients who receive transfusions may be humoral. Another study suggested that adenine and some unknown factors in the liquid or plasma portion of stored blood contributed to the inhibition of a normal lymphocytic proliferative response.[5]

The study by Busch et al. lends support to the theory that factors generated during the storage and processing of blood are responsible for the immunologic sequelae of perioperative blood transfusion in patients with cancer.

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To the Editor:

Busch et al. found that the use of autologous blood as compared with allogeneic blood for transfusion does not improve the prognosis of patients with colorectal cancer. Few details are presented on the number of patients receiving a given dose of autologous or allogeneic blood or other components. No details are provided on the receipt of blood components such as fresh-frozen plasma, platelets, and albumin, nor was it revealed whether such components were autologous or allogeneic. Does the group of recipients of autologous transfusions mentioned in Table 4 of the article include patients who received allogeneic plasma, platelets, or albumin?

We also wonder whether allogeneic red-cell transfusions were leukocyte-depleted in any manner at the blood center or hospital. Buffy-coat depletion is commonly used by the Dutch Red Cross, which supplies allogeneic blood to 14 of the 15 hospitals in the study. Leukocytes are suspected to be mediators of transfusion-induced immunomodulation on the basis of studies of renal-transplant recipients and of animals.[6,7] Leukocyte-depleted blood may diminish any immunosuppressive effect of transfusions.

Did the authors investigate the total transfused dose of various allogeneic or autologous components as a prognostic factor in tumor recurrence, given that the dose is a strong predictor in outcome? The administration of one to two units of allogeneic red cells alone may be insufficient to render a transfusion effect readily detectable.[8]

Another study of autologous transfusion and cancer recurrence is cited in the discussion as supporting the conclusions of this study.[9] The citation is to an abstract in which the authors conclude "blood transfusions have a relevant impact on recurrence rates in colorectal tumor patients." This reference appears to be cited in contradiction to its actual conclusions. The effect of transfusion on tumor recurrence remains an open question.

The authors reply:

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To the Editor:

We agree with Ross that autologous blood transfusions may induce the same deleterious effects as allogeneic transfusions. This should be considered as an alternative explanation for a noncausal relation between blood transfusions and prognosis in colorectal cancer. However, as we discussed in our paper, in several studies in animals, including our own,[10] syngeneic transfusions did not enhance tumor growth, whereas allogeneic transfusions did. It is interesting to consider the possibility that humoral factors in fresh-frozen plasma have an effect similar to that of blood transfusions. In our study, the number of patients who received allogeneic fresh-frozen plasma was too small to permit reliable conclusions about their prognostic impact. In the group of patients who received autologous blood transfusions, nearly half also received autologous plasma, whereas none received allogeneic plasma. In the group of patients who received one or two allogeneic transfusions, 9 received fresh-frozen plasma, whereas 66 did not. The rates of disease-free survival at 4 years were 69% in the former group and 58% in the latter (p=0.70). Of the patients who did not need transfusions, 129 received no plasma, 9 received allogeneic plasma, and 5 received autologous plasma. The rates of disease-free survival in these three groups at 4 years were 75%, 89%, and 40%, respectively (p=0.75).

Blumberg and Heal ask about possible dose effects of transfusions. We found no relation between the rate of recurrence of colorectal cancer and the number of transfusions.[11] In response to the argument that the transfusion of one to two units alone might be insufficient to have an effect, we found a transfusion effect of the same magnitude as the effect reported in a recent meta-analysis.[12]

The blood products used in our study were buffy-coat depleted, but not extra depleted of leukocytes. Such products could indeed overcome the detrimental effects of standard allogeneic transfusions whenever such effects exist.

We think that the study by Heiss et al.[9] supports our conclusions. The outcomes, as shown in life-table analyses (unfortunately not presented in their abstract but presented at conferences), were similar to those we reported.

Finally, we would like to emphasize that we performed a clinical trial that was not designed to investigate which substances in blood could be responsible for the putative immunologic changes.

References

1. Busch ORC, Hop WCJ, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 328:1372-6.
2. Marsh J, Donnan PT, Hamer-Hodges DW. Association between transfusion with plasma and the recurrence of colorectal carcinoma. Br J Surg 1990; 77:623-6.
3. Hermanek P Jr, Guggenmoos-Holzmann I, Schricker KT, et al. Der Einfluss der Transfusion von Blut und Haemoderivaten auf die Prognose des Colorectalen Carcinoms. Langenbecks Arch Chir 1989; 374:118-24.
4. Ross WB, Leaver HA, Yap PL, Raab GM, Su BH, Carter DC. Prostaglandin E_2 production by rat peritoneal macrophages: role of cellular and humoral factors in vivo in transfusion-associated immunosuppression. FEMS Microbiol Immunol 1990; 2:321-5.
5. Vliet WC, Dock NL, Davey FR. Factors in the liquid portion of stored blood inhibit the proliferative response in mixed lymphocyte cultures. Transfusion 1989; 29:41-5.
6. Blumberg N, Triulzi DJ, Heal JM. Transfusion-induced immunomodulation and its clinical consequences. Transfus Med Rev 1990; 4:Suppl 1:24-35.
7. Blajchman MA, Bardossy L, Carmen R, Sastry A, Singal DP. Allogeneic blood transfusion-induced enhancement of tumor growth: two animal models showing amelioration by leukodepletion and passive transfer using spleen cells. Blood 1993; 81:1880-2.
8. Blumberg N, Heal J, Chuang C, Murphy P, Agarwal M. Further evidence supporting a cause and effect relationship between blood transfusion and cancer recurrence. Ann Surg 1988; 207:410-5.
9. Heiss MM, Jauch KW, Delanoff Ch, Mempel W, Schildberg FW. Blood transfusion modulated tumor recurrence; a randomized study of autologous versus homologous blood transfusion in colorectal cancer. Proc Am Soc Clin Oncol 1992; 11:172. abstract.
10. Singh SK, Marquet RL, Westbroek DL, Jeekel J. Enhanced tumor growth of artificial tumor metastases following blood transfusion: the effect of erythrocytes, leukocytes, and plasma transfusion. Eur J Cancer Clin Oncol 1987; 23:1537-40.
11. Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Prognostic impact of blood transfusions on disease-free survival in colorectal carcinoma. Scand J Gastroenterol 1993; 28: Suppl 200:21-3.
12. Chung M, Steinmetz OK, Gordon PH. Perioperative blood transfusion and outcome after resection for colorectal carcinoma. Br J Surg 1993; 80:427-32.

CHAPTER 3

PROGNOSTIC IMPACT OF BLOOD TRANSFUSIONS ON DISEASE-FREE SURVIVAL IN COLORECTAL CARCINOMA

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Published in:
Scandinavian Journal of Gastroenterology 1993; 28: Suppl 200: 21-23.

Chapter 3

Summary

Blood transfusions have been indicated as having an adverse effect on the prognosis of patients treated surgically for cancer. We carried out a randomized trial to investigate whether a predeposit autologous blood transfusion program improved prognosis in patients with colorectal cancer as compared to the current practice of allogeneic transfusion. This appeared not to be the case. However, the subgroup of untransfused patients had a significantly better disease-free survival as compared with transfused patients; 73% and 59%, respectively (p=0.001). We found that the risk of recurrence was significantly increased for patients transfused with allogeneic, or with autologous, or with both types of blood, compared with those patients who did not require transfusions; relative recurrence rates were 2.3 (p=0.001), 1.8 (p=0.044), and 2.5 (p=0.009), respectively; these three rates did not differ significantly from each other.

We conclude that it is not the blood transfusions themselves, but the circumstances that necessitate the transfusions that are the real determinants of prognosis.

Introduction

The immunosuppressive effect of blood transfusions has been clearly demonstrated in patients undergoing kidney transplantation.[1] Whether blood transfusions have a detrimental effect on the prognosis of patients operated for a malignancy has long been a matter of debate. Although a relationship between blood transfusions and poor prognosis in colorectal cancer patients has been found in many retrospective studies,[2] conflicting results have been reported, too.[3] The question remains whether blood transfusions in themselves have a detrimental effect, or whether the relationship is only coincidental.[4] Randomization between transfused and untransfused patients is not possible,[5] and so comparison between these groups will always be biased by patient selection.[6]

We performed a prospective randomized trial to compare the effects of predeposit autologous blood transfusion with standard allogeneic blood transfusions on the prognosis of colorectal cancer. As reported recently, we did not find a difference in disease-free survival and survival between the two randomized groups.[7] The current study concerns a further analysis of this trial to determine whether the subgroup of untransfused patients has a different prognosis compared with the transfused group of patients.

Patients and methods

The material for this study was obtained from a randomized multicenter trial in which eligible patients were randomized equally into either the allogeneic or the autologous arm of the study. Patients were eligible if they were scheduled for a potentially curative resection of a colorectal carcinoma, and if they also fulfilled the criteria for autologous blood donation, as stated by the American Association of Blood Banks.[8] Patients in the autologous group were required to donate 450 ml of blood twice. The collected blood was separated into packed red cells and fresh-frozen plasma. All patients were operated by standard procedures and the rules for transfusion were standardized in both groups. Transfusion was allowed to be given when blood loss was more than 500 ml, or if the hemoglobin concentration dropped below 6.5 mmol/l. Operative specimens were staged according to the Turnbull modification[9] of the original Dukes' classification. Only patients who had a curative resection were taken into account for this study.

A standard follow-up program was used (including history, physical examination, and laboratory tests) every three months during the first two years and every six months thereafter. Chest X-ray and colonoscopy were performed yearly, and ultrasonography of the liver was done twice a year the first three years and once a year thereafter. Characteristic changes at physical examination or on chest X-ray, ultrasonography or CT-scan were accepted as evidence for recurrence. Metastatic or recurrent disease was confirmed by histologic or cytologic examination when possible.

Chi-square test and Mann-Whitney's test were used where appropriate. Cumulative 4 years' survival and disease-free survival were calculated using the life-table method of Kaplan-Meier.[10] The log-rank test was used to compare differences in Kaplan-Meier estimates. The Cox proportional-hazards models were used to evaluate factors in multivariate analysis.[11] Two-sided p values ≤0.05 were considered statistically significant.

Results

A total of 423 patients with colorectal carcinoma were operated curatively. In the allogeneic group there were 216 patients and 207 in the autologous group. Patient characteristics did not differ significantly between the two groups. Comparisons between the patients receiving transfusions and those who did not are shown in Table 3.1.

Table 3.1. Comparison between transfused and untransfused patients.

	Untransfused (N = 143)		Transfused (N = 280)		p value
Age (years)	65	(33-88)	67	(31-88)	0.049
Sex					
Male	85	(59%)	158	(56%)	n.s.
Female	58	(41%)	122	(44%)	
Operation					
Right hemicolectomy	25	(17%)	22	(8%)	<0.0001
Transverse colectomy	4	(3%)	3	(3%)	
Left hemicolectomy	12	(8%)	17	(6%)	
Sigmoid resection	47	(33%)	40	(14%)	
Anterior resection	48	(34%)	118	(42%)	
Abdominoperineal resection	6	(4%)	80	(29%)	
Subtotal colectomy	1	(1%)	-	-	
Dukes' classification					
A	41	(29%)	67	(24%)	n.s.
B	50	(35%)	115	(41%)	
C	52	(36%)	98	(35%)	
Histological differentiation					
Well	19	(13%)	43	(15%)	n.s.
Moderate	111	(78%)	203	(73%)	
Poor	13	(9%)	32	(11%)	
Adjacent organ fixation	7	(5%)	25	(9%)	n.s.
Tumor size (cm)	4.0	(0.5-15.0)	4.4	(0.8-10.0)	0.018
Blood loss (ml)	400	(100-1,500)	1100	(100-11,500)	<0.0001

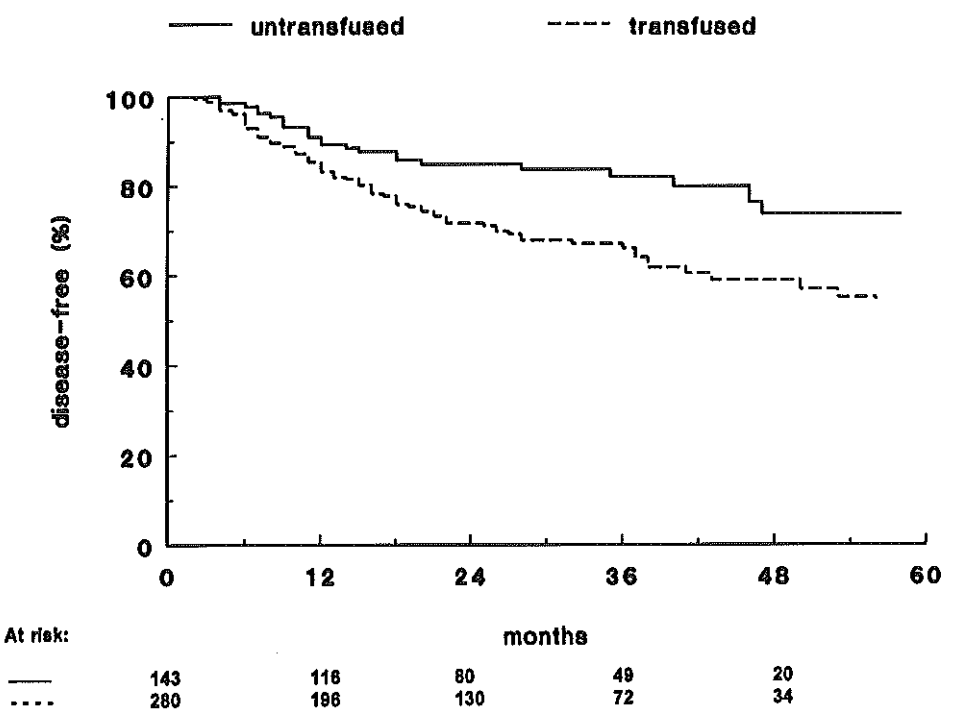
Continuous data are shown as medians with ranges in parentheses, and categorical data as numbers of patients with percentages in parentheses.

In the transfused group the patients were older, had larger tumors and the amount of blood loss was significantly greater. In addition, there were significantly more anterior resections and abdominoperineal resections in the transfused group compared with the untransfused group.

In the untransfused group 24 out of 143 patients developed recurrent disease as compared with 81 out of the 280 patients in the transfused group. The disease-free survival at 4 years was 73% in the untransfused group, and 59% in the transfused group (p=0.001) (Figure 3.1).

Figure 3.1. Disease-free survival comparing untransfused with transfused patients.

The disease-free survival at 4 years is 73% in the untransfused group and 59% in the transfused group (p=0.001).



In Cox regression analysis, allowing for various prognostic factors the Dukes' classification was a very strong prognostic factor (Table 3.2). Blood transfusion was also correlated with poor prognosis. Randomization, however, was not a predictor of prognosis.

Table 3.2. Multivariate analysis of various factors with respect to disease-free survival of the patients who underwent curative surgery.

Factor	Relative recurrence rate	95% confidence interval	p value
Randomization			
Allogeneic group	1	-	-
Autologous group	0.9	0.6 - 1.4	0.671
Age*	1.0	1.0 - 1.0	0.848
Sex			
Male	1	-	-
Female	1.2	0.8 - 1.7	0.478
Dukes' classification			
A	1	-	-
B	3.8	1.6 - 9.1	0.003
C	10.1	4.8 - 20.7	<0.001
Tumor size*	1.1	1.0 - 1.2	0.061
Differentiation			
Well	1	-	-
Moderate	1.2	0.6 - 2.5	0.550
Poor	2.1	0.9 - 4.7	0.700
Operation			
Intra-abdominal	1	-	-
Rectal involvement	1.1	0.7 - 1.7	0.671
Adjacent organ fixation			
No	1	-	-
Yes	0.4	0.2 - 0.9	0.027
Transfusions			
No	1	-	-
Yes	2.1	1.3 - 3.5	0.004

* As compared to 1 year younger (age) or 1 cm smaller (tumor size).

The group of transfused patients was subdivided into those who received only allogeneic (N=136), only autologous (N=102) and those receiving both types of transfusions (N=42) (Figure 3.2). At 4 years the disease-free survival was 56%, 62%, and 66%, respectively (p=0.50). The recurrence rates of the different types of transfusions were all significantly elevated as compared with those of the untransfused patients in Cox regression analysis adjusting for Dukes' classification (Table 3.3). There was no difference between the relative recurrence rates for the different types of transfusions (p=0.49).

Figure 3.2. Disease-free survival comparing untransfused patients with different types of transfused patients.

The group of patients receiving only allogeneic transfusions (allo), only autologous transfusions (auto), and both types of transfusions (both) are all significantly (p=0.007) different from the untransfused patients (none).

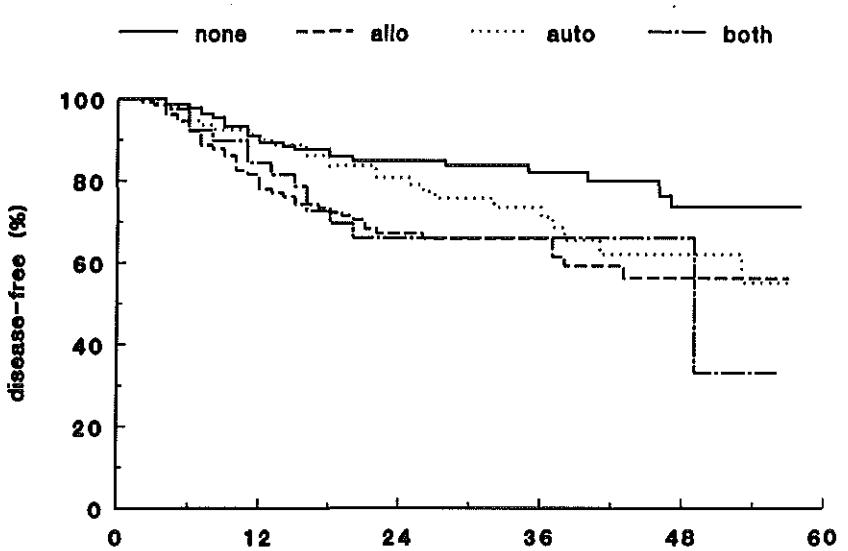


Table 3.3. Multivariate analysis for Dukes' classification and transfusion status.

Factor	Relative recurrence rate	95% confidence interval	p value
Dukes' classification			
A	1	-	-
B	3.8	1.6 - 9.1	0.002
C	10.9	4.7 - 25.2	<0.001
Transfusions			
None	1	-	-
Allogeneic	2.3*	1.4 - 3.8	0.001
Only autologous	1.8*	1.0 - 3.1	0.044
Allogeneic and autologous	2.5*	1.3 - 4.8	0.009

* No significant difference between the three transfusion groups (p=0.49).

Discussion

This analysis shows that there is a relation between blood transfusions and disease-free survival in colorectal cancer patients. The relationship, however, is not likely to be causal, because the increased relative risk is about the same between those patients receiving allogeneic transfusions, those receiving only autologous transfusions, and those receiving both types of transfusions.

As reported earlier, the implementation of an autologous blood donation program reduced the exposure to allogeneic blood considerably,[12] but had no advantage in the prognosis of colorectal cancer patients.[7]

Similarly, Tartter,[13] in a prospective study, showed a significant difference between the untransfused and transfused patients; the 5 years' disease-free survival was 77% for untransfused patients and 57% for transfused patients. In a recent meta-analysis,[14] including 20 retrospective studies representing over 5000 patients, a relationship between blood transfusions and poor prognosis of the same magnitude as our results was reported. The cumulative odds-ratio of the disease-free survival was 1.8.

We conclude that blood transfusions are related to poor prognosis in colorectal cancer patients, but this relationship is not causal. Therefore, it is not the blood transfusions themselves but rather the circumstances that necessitate transfusion that are the real determinants of prognosis.

References

1. Opelz G, Sengar DPS, Mickey MR, Terasaki PI. Effect of blood transfusion on subsequent kidney transplants. Transplant Proc 1973; 5:253-9.
2. Blumberg N, Heal JM. Transfusion and host defenses against cancer recurrence and infection. Transfusion 1989; 29:236-45.
3. Francis DMA. Relationship between blood transfusion and tumour behaviour. Br J Surg 1991; 78:1420-8.
4. Brunson ME, Alexander JW. Mechanisms of transfusion-induced immunosuppression. Transfusion 1990; 30:651-8.
5. van Lawick van Pabst WP, Langenhorst BLAM, Mulder PGH, Marquet RL, Jeekel J. Effect of perioperative blood loss and perioperative blood transfusion on colorectal cancer survival. Eur J Cancer Clin Oncol 1988; 24:741-7.
6. Taylor RMR, Parrott NR. Red alert. Br J Surg 1988; 75:1049-50.
7. Busch ORC, Hop WCJ, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 328:1372-6.
8. Council on Scientific Affairs. Autologous blood transfusions. JAMA 1986; 256:2378-80.
9. Turnbull RB, Kyle K, Watson FR, Spratt J. Cancer of the colon: The influence of the no-touch isolation technic on survival rates. Ann Surg 1967; 166:420-5.
10. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457-81.
11. Cox DR. Regression models and life tables. J R Stat Soc [B] 1972; 34:187-202.
12. Hoynck van Papendrecht MAW, Hop W, Langenhorst BLAM, Kothe FC, Marquet RL, Jeekel J. Feasibility of a predeposit autologous blood donation program in colorectal cancer patients: Results from a randomized clinical study. Vox Sang 1992; 62:102-7.
13. Tartter PI. The association of perioperative blood transfusion with colorectal cancer recurrence. Ann Surg 1992; 216:633-8.
14. Chung M, Steinmetz OK, Gordon PH. Perioperative blood transfusion and outcome after resection for colorectal carcinoma. Br J Surg 1993; 80:427-32.

CHAPTER 4

BLOOD TRANSFUSIONS AND LOCAL TUMOR RECURRENCE IN COLORECTAL CANCER: EVIDENCE OF A NONCAUSAL RELATIONSHIP

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Published in:
Annals of Surgery 1994; 220: 791-797.

Chapter 4

Summary

Retrospective studies suggest that blood transfusions are associated with a poor prognosis in patients who undergo operations for colorectal malignancies. In a previously published, randomized trial, it was investigated whether autologous blood transfusions could overcome this putative detrimental effect. However, this did not appear to be the case. In the current study we analyzed the patterns of recurrence in 420 patients who underwent curative operations for colorectal cancer. Patients who did not require transfusions (N=143) had significantly better disease-free survival than those who did need transfusions (N=277); percentages at 4 years were 73% and 59%, respectively (p=0.001). No difference was found between both groups in comparing cumulative percentages of patients having metastases; percentages at 4 years were 25% in the group that did not undergo transfusion and 27% in the transfused group. The percentage of cases having local recurrence, however, was significantly increased (p=0.0006) in the transfused group as compared with the group that did not undergo transfusion; percentages at 4 years were 20% and 3%, respectively. The groups of patients receiving only allogeneic, only autologous, or both types of transfusions all had a significantly higher incidence of local recurrence than the patients who did not receive transfusions, but no differences were found between these three groups. These findings suggest that the association between blood transfusions and prognosis in colorectal cancer is a result of the circumstances that necessitate transfusions, leading to the development of local recurrences but not of distant metastases.

Introduction

It has been suggested that allogeneic blood transfusions are associated with a poor prognosis of patients who undergo operations for colorectal cancer, possibly because of immunologic factors.[1,2,3,4] A recent meta-analysis, combining the evidence of 20 published retrospective studies, demonstrated that transfused patients with colorectal cancer generally had a worse prognosis when compared with patients who did not undergo transfusion.[5] However, in a randomized trial of 475 patients, we did not find that patients transfused with autologous blood had a better prognosis than those patients transfused with allogeneic blood, although transfused patients, receiving either type of blood, had a poorer prognosis than patients who did not receive transfusions.[6] Therefore, the circumstances necessitating transfusions rather than the blood transfusions themselves are the real determinant of prognosis. Naturally, the need for postoperative transfusions was associated with the amount of blood loss. This might be related to technical difficulties to resect the tumor, and to surgical skill.[7] These factors also may affect the development of local recurrence, because local recurrences originate from remaining viable local tumor residues,[8] or local spill.[9] Previous studies reporting on the effects of blood transfusions on prognosis presented data on survival or disease-free survival. However, in only a few studies the incidence of local recurrence and metastatic disease was evaluated separately, but did not come to conclusions.[10] In the current study, the relationship between blood transfusions and the patterns of recurrent disease in patients participating in the aforementioned trial was investigated.

Methods

Those evaluated were patients who underwent curative operations and participated in a randomized, multicenter trial to investigate the effect of autologous blood on prognosis of colorectal cancer as compared with standard allogeneic transfusions. The design of this trial has been described in detail elsewhere.[6] Briefly, patients with a potentially curative resection of a colorectal carcinoma were eligible if they fulfilled the criteria set for autologous blood donation.[11] Patients randomized into the autologous group had to donate two units of blood in two sessions before operation. The collected blood was separated into packed red cells and fresh-frozen plasma. The transfusion rules were the same for patients in the autologous group as for patients in the allogeneic group. Packed red cells were allowed to be given if blood loss exceeded 500 ml or if the hemoglobin level dropped below 10.5 g/dl.

After standard surgical procedures, the tumors were staged according to the Turnbull[12] modification of the original Dukes' classification. A tumor confined to the bowel wall was staged as Dukes' A; if the tumor extended through the serosa into the pericolic fat, it was

staged as Dukes' B: and if regional lymph nodes contained metastases, it was staged as Dukes' C. En bloc resected tumors with adjacent organ fixation were not staged as Dukes' D. No adjuvant chemotherapy was given, and pelvic radiation was only given in a minority of cases.

A standard follow-up program was used, including history, physical examination, and laboratory tests, every three months during the first two years and every six months thereafter. Chest X-ray and colonoscopy were performed yearly, and ultrasonography of the liver was done twice a year for the first three years and once a year thereafter.

If possible, histologic or cytologic evidence was obtained to confirm metastatic or local recurrent disease. Characteristic changes at physical examination, on X-ray, on liver ultrasonography, or on computer tomography scan also were accepted as metastatic disease or as local recurrence.

In this study, the incidence of distant metastases and local recurrence were analyzed as first signs of recurrent disease. Of the 475 randomized patients, 423 patients underwent curative surgery. Three of these patients died from postoperative complications. The remaining 420 patients form the basis of this report.

Incidences implicating cumulative percentages of patients having metastases and cumulative percentages of patients having local recurrences were calculated according to the Kaplan-Meier method.[13] The log-rank test was used to compare these estimates. Multivariate analyses were performed using Cox regression.[14] Two-sided p value ≤ 0.05 was considered the limit of statistical significance.

Results

Patient characteristics

Of the 420 patients, 214 patients belonged to the allogeneic group and 206 to the autologous group. The median follow-up period of the patients was 2.3 years (range, 1 to 59 months). No patient was lost to follow-up.

Of the 420 patients, 277 received transfusions and 143 patients did not. Of these 277 patients, 134 received only allogeneic transfusions, 101 received only autologous transfusions, and 42 patients received both types of transfusions.

Recurrent disease

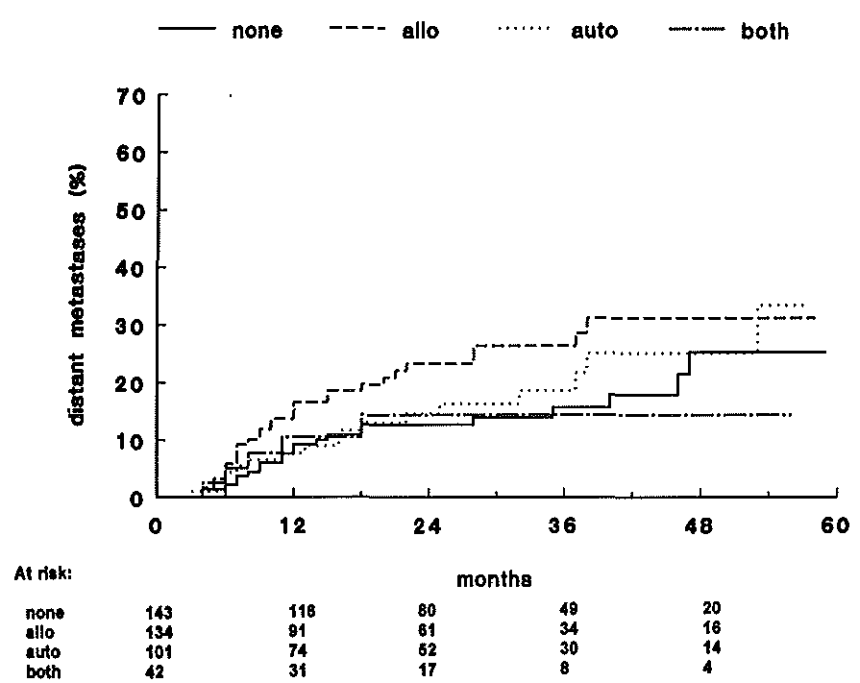
For all studied patients (i.e., whether they received transfusions or not) the disease-free survival at 4 years was 66% in the allogeneic group and 63% in the autologous group (p=0.93). In the allogeneic group, 54 of 214 patients developed recurrent disease (39 distant metastases, 13 local recurrences and two patients both simultaneously). In the autologous group, 51 of 206 patients developed recurrent disease (30 distant metastases, 20 local recurrences and one patient both). The distribution of sites of recurrent disease per randomized group is shown in Table 4.1.

Table 4.1. First detected site of recurrent disease according to randomized group.

	Allogeneic group (N = 214)		Autologous group (N = 206)	
Total	54		51	
Local recurrence	13		20	
Metastatic disease	39		30	
Liver	23	(59%)	17	(57%)
Lung	5	(13%)	2	(7%)
Brains	2	(5%)	-	-
Other	3	(8%)	5	(16%)
Multiple	6	(15%)	6	(20%)
Local and metastatic disease	2		1	

Figure 4.1. Cumulative percentages of patients having distant metastases according to transfusions received.

The group of patients receiving only allogeneic transfusions (allo), only autologous transfusions (auto), and both types of transfusions (both) are all not significantly different from the untransfused patients (none).



The disease-free survival at 4 years was 59% in the group of 277 patients who received transfusions versus 73% in the group of 143 patients who did not require transfusions (p=0.001). Using multivariate analysis, allowing for various prognostic factors, blood transfusion was a significant determinant of disease-free survival.[15]

Table 4.2. Characteristics of patients with metastatic disease and with local recurrence.

	Total	Metastatic disease	Local recurrence
Randomization			
Allogeneic group	214	39 (41)	13 (15)
Autologous group	206	30 (31)	20 (21)
Age (years)	67	67 (67)	66 (66)
Sex			
Male	242	39 (42)	12 (15)
Female	178	30 (30)	21 (21)
Operation			
Intra-abdominal	171	30 (30)	7 (7)
Rectal involvement	249	39 (42)	26 (29)
Dukes' classification			
A	107	2 (2)	4 (4)
B	165	23 (24)	13 (14)
C	148	44 (46)	16 (18)
Histological differentiation			
Well	62	6 (7)	2 (3)
Moderate	311	47 (49)	27 (29)
Poor	45	16 (16)	4 (4)
Tumor size (cm)	4	4 (4)	5 (5)
Adjacent organ fixation	32	4 (4)	3 (3)
Adjuvant irradiation	31	11 (12)	5 (6)
Blood loss (ml)	750	900 (900)	1300 (1300)
Blood transfusions			
No	143	21 (21)	3 (3)
Yes	277	48 (51)	30 (33)

Continuous data are presented as medians and categorical data as numbers of patients.
Adding those patients with both metastatic disease and local recurrence are shown in parentheses.

The characteristics of the patients with metastatic disease and those with local recurrences are shown in Table 4.2, and the univariate evaluations of different prognostic factors are reported in Table 4.3. No statistically significant differences were found in the cumulative percentages of distant metastases and of local recurrence in comparing the randomized groups. The Dukes' classification was a significant prognostic factor for both end points.

The grade of differentiation also was a significant factor for the incidence of metastases, but in Cox regression analysis, allowing for Dukes' stage, this was not the case. Involvement of the rectum, blood loss, and blood transfusions each were related significantly with the incidence of local recurrence, but not with the incidence of metastatic disease (Table 4.3).

Table 4.3. Univariate comparisons of patients having distant metastases and those having local recurrence according to various factors.

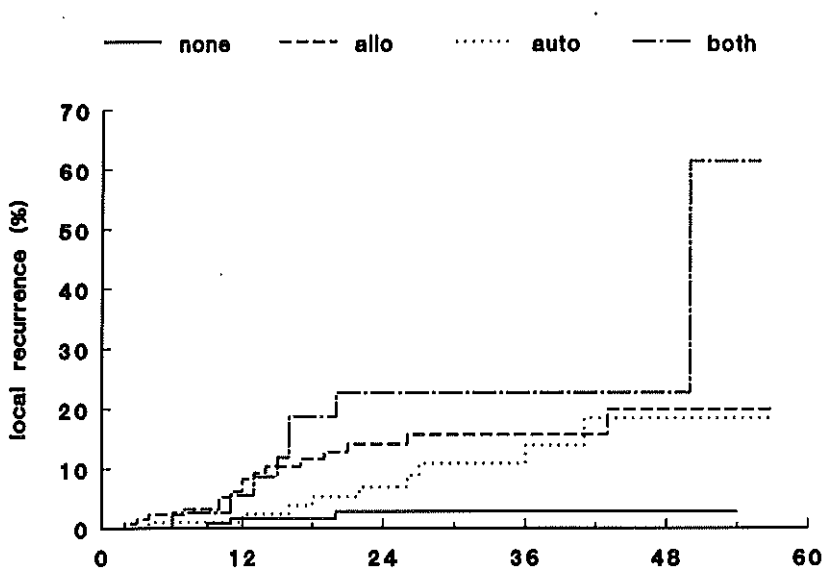
	Metastases*	log-rank p value	Local recurrence*	log-rank p value
Randomization				
Allogeneic group	27%	n.s.	11%	n.s.
Autologous group	25%		17%	
Age (years)				
≤ 65	27%	n.s.	13%	n.s.
> 65	27%		14%	
Sex				
Male	29%	n.s.	12%	n.s.
Female	22%		16%	
Operation				
Intra-abdominal	27%	n.s.	7%	0.007
Rectal involvement	25%		19%	
Dukes' classification				
A	2%	<0.0001	7%	0.004
B	23%		10%	
C	49%		27%	
Differentiation				
Well	20%	0.0006	7%	n.s.
Moderate	24%		14%	
Poor	49%		25%	
Tumor size (cm)				
< 5	26%	n.s.	13%	n.s.
≥ 5	26%		16%	
Adjacent organ fixation				
No	27%	n.s.	14%	n.s.
Yes	13%		8%	
Blood loss (ml)				
≤ 500	23%	n.s.	4%	0.001
> 500 and ≤ 1000	28%		14%	
> 1000	28%		24%	
Blood transfusions				
No	25%	n.s.	3%	0.0006
Yes	27%		20%	

* Cumulative percentages at 4 years, according to Kaplan-Meier estimates.

Cumulative percentages of metastases comparing the patients who did not undergo transfusion and the transfused patients is shown in Figure 4.1. The percentage of metastases at 4 years was 25% in the group of patients who did not undergo transfusion and 27% in the transfused group. The percentages of metastases did not differ significantly between the different types of transfusions; at 4 years, the cumulative percentage of metastases in the group of patients receiving only allogeneic transfusions (N=134), only autologous transfusions (N=101) or both types of blood transfusions (N=42) were 31%, 33%, and 14%, respectively.

Figure 4.2. Cumulative percentages of patients having local recurrence according to transfusions received.

The group of patients receiving only allogeneic transfusions (allo), only autologous transfusions (auto), and both types of transfusions (both) are all significantly different from the untransfused patients (none).



However, the cumulative percentage of local recurrence was significantly less in the group of patient who did not undergo transfusion as compared with the groups of patients with different types of transfusions (Figure 4.2). The percentage of local recurrence was 3% in the group that did not undergo transfusion and 20% in the transfused group (p=0.0006). The cumulative percentages of local recurrence at 4 years in the group of patients receiving only allogeneic transfusions, only autologous transfusions, or both types of blood transfusions were 20%, 18%, and 23%, respectively, and all were significantly higher than the group that did not undergo transfusion (p=0.001, p=0.02, and p<0.001, respectively). The percentages of local recurrence of these three transfused groups of patients did not significantly differ between each other (p=0.18).

Multivariate analyses, not allowing for blood loss, showed that blood transfusion was a significant determinant for local recurrences. This was not the case for metastases (Table 4.4). When postoperative radiation, given in a minority of cases (Table 4.2), also was taken into account in these regression models, the estimates and p values did not change appreciably, and irradiation was not an additional prognostic factor. When blood loss in the multivariate analysis was taken into consideration, the impact of blood transfusions on the local recurrence rate was no longer statistically significant (Table 4.4).

To explore the effect on prognosis of preoperative autologous donation itself, only the patients who did not undergo transfusion were evaluated. Comparison of those patients in the allogeneic group (N=94) with those in the autologous group (N=49) showed no significant differences in disease-free survival (75% versus 69%), percentage of distant metastases (23% versus 29%), and percentage of local recurrence (3% versus 2%).

Table 4.4. Multivariate analysis of the incidence of metastases and local recurrence.

Factor	Relative metastases rate	p value	Relative local recurrence rate	p value
Blood transfusions				
No	1	-	1	-
Yes	1.6	n.s.	5.2*	0.008
Dukes' stage				
A	1	-	1	-
B	7.6	0.006	2.2	n.s.
C	23.8	<0.001	5.1	0.004
Operation				
Intra-abdominal	1	-	1	-
Rectal involvement	1.0	n.s.	2.0	n.s.

* This estimate is 3.5 (p=0.06) when in the analysis also blood loss was taken into account (relative local recurrence rate equals 1.3 as compared with 50% lower amount of blood loss; p=0.14). The addition of blood loss did not appreciably change the other estimates or their p values.

Discussion

Blood transfusions seem to be associated with poor prognosis of colorectal cancer. This relationship appeared not to be causal; rather, the circumstances that necessitate transfusions are of prognostic importance in colorectal cancer patients.[6] Of all studies reporting on recurrent disease from colorectal cancer and blood transfusions, only a few made a distinction between local recurrence and metastatic disease in the analyses. So far, no additional information has been given about the negative association between blood transfusions and prognosis.

The current study shows that the relationship between blood transfusions and the increased risk of recurrent disease is a result of an increased risk of local recurrences. No relation was found with the incidence of distant metastases. Similar findings applied to the amount of blood loss, but evaluating blood loss and transfusions simultaneously by multivariate analysis made the significance of either factor disappear. This is a consequence of the strong relationship that existed between blood loss and transfusion because of our transfusion rules (median blood loss in case of no transfusion was 400 ml and in case of transfusion 1100 ml; $p<0.001$). The only other prognostic factor affecting local recurrence and not metastatic disease was an operation of a rectal tumor, compared with an intra-abdominal tumor. Although resections of rectal tumors are associated with larger amounts of blood loss and therefore, require more transfusions, multivariate analysis showed blood transfusion to be an independent factor of prognosis. In addition, there are studies restricted to colonic cancer that found a detrimental effect of blood transfusions, too.[16,17]

In the current study, we found that the influence of rectal involvement was not additional to the effect of blood transfusions in multivariate analysis. In our patient population, the incidence of rectal involvement was relatively high, which is caused by our inclusion criteria. All patients should be able to donate blood and therefore, must have a hemoglobin level of at least 11.3 g/dl (7 mmol/l). Patients with tumors in the right colon often have had anemia or transfusions preoperatively and are (in both situations) ineligible for our study.

In this study, only three patients had both local recurrence and metastatic disease at the moment recurrent disease was diagnosed. In retrospective studies, this number usually was higher, which probably can be explained by the prospective design of our study and our intensive follow-up program, in which examinations such as liver ultrasonography were performed routinely.

From the moment recurrent disease was detected, the median survival time was similar in patients who had local recurrences and those who had distant metastases. Thus, a detrimental prognostic relationship between transfusions and the development of local recurrence will have a comparable effect on survival. The retrospective studies reporting on the effect of blood transfusions and cancer survival might, therefore, be explained by a deleterious relationship of transfusions and local recurrence only.

A recent prospective study by Tartter[18] found a transfusion effect on the disease-free survival of colorectal cancer patients. The 5 years' disease-free survival was 77% for patients who did not undergo transfusion and 57% for transfused patients. In a recent meta-analysis,[5] representing over 5000 patients, a relationship between blood transfusions and poor prognosis was reported of the same magnitude as found in our study. Unfortunately, in those studies, there were no data available on the incidence of local recurrence and the incidence of metastatic disease separately.

The fact that local recurrence represents failure of the surgical technique is demonstrated by McArdle and Hole,[19] who reported on the variability among surgeons on postoperative complications and ultimate survival. It has been suggested that a more meticulous and careful dissection of the pararectal tissues reduces the incidence of local recurrence for rectal tumors.[20] Recently, the results of total mesorectal excision for rectal cancer were reported and showed that such surgery gave better disease-free survival than other studies had demonstrated using adjuvant radiation or chemotherapy.[21,22]

Another explanation of the current findings might be the presence of growth factors released from platelets after blood loss in the peritoneal cavity. An enhanced peritoneal tumor load was found in rats receiving serum intraperitoneally as compared with those that did not receive it.[23] However, the rate of liver metastases also was increased in this animal model.

The current study shows that the prognostic association between blood transfusions and colorectal cancer is mainly a result of the an increased risk of local recurrences and not an increased risk of metastatic disease. Assuming that local recurrence and the need for blood transfusions are related to surgical difficulties and skill, operations on patients with colorectal cancer should be performed in a meticulous way, with precise tumor excision and as few transfusions as necessary.

Therefore, patients who are scheduled for potentially curative resections of colorectal malignancies may have better prognoses if their surgeries are performed by surgeons who are experienced in colorectal cancer surgery.

References

1. Gantt CL. Red blood cells for cancer patients. Lancet 1981; 2:363.
2. Burrows L, Tartter P. Effect of blood transfusions on colonic malignancy recurrence rate. Lancet 1982; 2:662.
3. Francis DMA. Relationship between blood transfusion and tumour behaviour. Br J Surg 1991; 78:1420-8.
4. Blumberg N, Heal JM. Transfusion and host defenses against cancer recurrence and infection. Transfusion 1989; 29:236-45.
5. Chung M, Steinmetz OK, Gordon PH. Perioperative blood transfusion and outcome after resection for colorectal carcinoma. Br J Surg 1993; 80:427-32.
6. Busch ORC, Hop WCJ, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 328:1372-6.
7. Phillips RKS, Hittinger R, Blesovsky L, Fry JS, Fielding LP. Local recurrence following 'curative' surgery for large bowel cancer: I. The overall picture. Br J Surg 1984; 71:12-6.
8. Heald RJ, Ryall RDH. Recurrence and survival after mesorectal excision for rectal cancer. Lancet 1986; 1:1497-82.
9. Quirke P, Durdey P, Dixon MF, Williams NS. Local recurrence of rectal adenocarcinoma due to inadequate surgical resection. Histopathological study of lateral tumour spread and surgical excision. Lancet 1986; 2:996-8.
10. Cheslyn-Curtis S, Fielding LP, Hittinger R, Fry JS, Phillips RKS. Large bowel cancer: the effect of perioperative blood transfusion on outcome. Ann R Coll Surg Engl 1990; 72:53-9.
11. Council on Scientific Affairs. Autologous blood transfusion. JAMA 1986; 256:2378-80.
12. Turnbull RB, Kyle K, Watson FR, Spratt J. Cancer of the colon: The influence of the no-touch isolation technic on survival rates. Ann Surg 1967; 166:420-5.
13. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457-81.
14. Cox DR. Regression models and life tables. J R Stat Soc [B] 1972; 34:187-202.
15. Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Prognostic impact of blood transfusions on disease-free survival in colorectal carcinoma. Scand J Gastroenterol 1993; 28 Suppl 200:21-3.
16. Agarwal M, Blumberg N. Colon cancer patients transfused perioperatively have an increased incidence of recurrence. Transfusion 1983; 23:421.
17. Voogt PJ, van de Velde CJH, Brand A, et al. Perioperative blood transfusion and cancer prognosis; Different effects of blood transfusion on prognosis of colon and breast cancer patients. Cancer 1987; 59:836-43.
18. Tartter PI. The association of perioperative blood transfusion with colorectal cancer recurrence. Ann Surg 1992; 216:633-8.
19. McArdle CS, Hole D. Impact of variability among surgeons on postoperative morbidity and mortality and ultimate survival. Br Med J 1991; 302:1501-5.
20. Cawthorn SJ, Parums DV, Gibbs NM, et al. Extent of mesorectal spread and involvement of lateral resection margin as prognostic factors after surgery for rectal cancer. Lancet 1990; 335:1055-9.
21. Heald RJ, Karanjia ND. Results of radical surgery for rectal cancer. World J Surg 1992; 16:848-57.
22. MacFarlane JK, Ryall RDH, Heald RJ. Mesorectal excision for rectal cancer. Lancet 1993; 341:457-60.
23. Lawrance RJ, Cooper AJ, Loizidou M, Alexander P, Taylor I. Blood transfusion and recurrence of colorectal cancer: the role of platelet derived growth factors. Br J Surg 1990; 77:1106-9.

CHAPTER 5

BLOOD TRANSFUSIONS AND POSTOPERATIVE INFECTIOUS COMPLICATIONS AFTER COLORECTAL CANCER SURGERY

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Submitted for publication.

Summary

In several studies it was suggested that blood transfusions may have a detrimental effect on the susceptibility to infectious complications after surgery. So far no large clinical study investigated this issue in a randomized setting. We analyzed the postoperative infectious complications of 470 patients who were operated for colorectal cancer and who had been randomized to receive allogeneic or autologous blood transfusions if required. The objective was to determine in a randomized multicenter clinical trial, whether predeposit autologous blood transfusions can decrease the incidence of postoperative infections. In the allogeneic group 58 of the 234 patients (25%) and in the autologous group 64 of the 236 patients (27%) had postoperative infectious complications ($p=0.64$). Regression analysis showed that tumor location, age and blood transfusion were significant risk factors for infections. Patients who received more than two units of blood had a significantly increased risk of infections, irrespective of the type of transfusions received, whereas patients receiving up to two transfusions had the same rate of infections as those patients who did not need transfusions. The odds-ratio for infections was 2.0 (95% confidence interval, 1.0 to 3.9; $p<0.001$) for patients receiving more than two allogeneic transfusions and 3.6 (95% confidence interval, 1.8 to 7.3; $p<0.05$) for patients receiving autologous and allogeneic transfusions. These two ratios were not significantly different from each other ($p=0.14$). We conclude that a predeposit autologous blood transfusion program does not decrease the risk of infectious complications after colorectal cancer surgery. We further believe that our finding of an increased risk of infections in case a large number of transfusions is given, is not due to the blood transfusions themselves, but rather the circumstances necessitating them.

Introduction

Several studies on the risk of infectious complications after colorectal cancer surgery have found that the incidence of postoperative bacterial infections is increased when patients were transfused.[1,2,3] The immunological changes induced by perioperative allogeneic blood transfusions are held responsible for this increased susceptibility to infections.[4,5] Obviously, blood transfusions are not the only immunomodulatory factors in the perioperative period. Also anesthesia, blood loss and surgical stress may influence the patients immune status. However most studies have found that after correcting for various risk factors of infections, by using multivariate analysis, blood transfusion was an independent factor.[6] Unfortunately, the causality of the factor blood transfusion is impossible to investigate properly in uncontrolled studies. The need for transfusion might be related to factors which are unknown or difficult to quantify and therefore unable to properly allow for. Surgical difficulty and skill could be related to both the need for blood transfusions and postoperative infectious complications. One of the options to overcome the possible detrimental effects of transfusions is the use of predeposit autologous blood, because no immunological changes induced by autologous transfusions have been reported so far.

We have performed a randomized multicenter study to compare the effects of allogeneic and autologous blood transfusions on prognosis and infectious complications after colorectal cancer surgery. The results on cancer prognosis have been published recently.[7] The present paper reports on the relationship between the different types of blood transfusions and postoperative infectious complications observed in this randomized clinical trial.

Methods

Patients who were scheduled for a curative resection of a colorectal carcinoma were eligible for the study if they fulfilled the criteria set for autologous blood donation.[8] Between August 1986 and November 1991, eligible patients in the 15 participating hospitals were randomly assigned either to the allogeneic group or to the autologous group. The randomization procedure included stratification per hospital. Patients randomized to the autologous group were required to donate two units of blood prior to surgery. These units of blood were separated into packed red cells (without buffy-coat) and fresh-frozen plasma. If needed according to our transfusion rules, blood loss over 500 ml or a hemoglobin concentration drop below 10.5 g/dl (6.5 mmol/l), the packed red cells were transfused. In the allogeneic group third party blood transfusions were given if necessary regarding the same rules for transfusion. The design of this trial has been described in more detail elsewhere.[7]

Bowel irrigation and antibiotic prophylaxis were given routinely in each hospital. Postoperative infectious complications were scored by the responsible surgeon. Wound infections were defined as drainage of pus from the wound, urinary tract infections as positive urine culture with clinical signs, pneumonia as positive sputum culture with specific clinical signs or changes on chest X-ray. Intra-abdominal infections were those found during laparotomy or abscesses which were drained transcutaneously by using ultrasonography or CT-scan.

A total of 475 patients were randomized in this trial. Five patients (two in the allogeneic group and three in the autologous group) were not operated after randomization and were excluded from the analysis.

Percentages were compared by the chi-square test (with Yates' correction in case of 2 by 2 tables). Tests for trend were performed when appropriate. Logistic regression[9] was used to investigate various factors simultaneously regarding the incidence of infectious complications. Exact 95% confidence limits of odds-ratios were determined using the program EGRET.[10] Two-sided p value ≤ 0.05 was considered statistically significant.

Results

The characteristics of the 470 patients operated for colorectal carcinoma were not greatly different comparing the two randomized groups (Table 5.1).

Eight patients (three in the allogeneic group and five in the autologous group) died of postoperative complications. In the allogeneic group 58 out of the 234 patients (25%) had postoperative infectious complications as compared with 64 of the 236 patients in the autologous group (27%). These percentages did not differ significantly from each other (p=0.64).

To investigate the influence of the multicentricity of our study we calculated the odds-ratios (autologous versus allogeneic randomized group) for infectious complications per hospital (Figure 5.1). By analyzing this hospital effect it was found that there was no significant difference (p=0.97) in odds-ratios for the 15 participating hospitals. The common odds-ratio for infections was 1.2 (95% confidence interval, 0.8 to 1.9), which did not differ significantly (p=0.49) from 1, i.e. the value indicating no difference between both randomized groups. Logistic regression analysis, including age and tumor location, was performed to obtain a higher level of precision in the comparison of the randomized groups. The adjusted odds-ratio for the risk of infectious complications in the autologous versus the allogeneic group was 1.2 (95% confidence interval, 0.8 to 1.8; p=0.48).

Comparing the different sites of infectious complications also did not show a significant difference between the two randomized groups (Table 5.2).

Univariate analyses regarding possible influencing factors are shown in Table 5.3. Location of the tumor in the rectum, and the age of the patient were the most important

factors associated with the risk of infections. Also blood loss and transfusions of fresh-frozen plasma were factors which were significantly related to the risk of infections, whereas blood transfusions (red cells) did not affect the infection rate. The group of patients receiving blood transfusions of any type had infections in 29% of the cases as compared with 21% in the group of patients who did not receive transfusions (p=0.084). However, when the group of patients who received transfusions were subdivided in different types of transfusions a significant difference for the rate of infectious complications appeared (p<0.0001). Transfusion of more than two units of blood increased the risk of infections markedly whereas the first two transfusions did not. Rates of infections were 21%, 19%, and 19% for the patients without transfusions, one or two allogeneic transfusions, and one or two autologous transfusions, respectively. The patients receiving more than two allogeneic transfusions had in 40% of the cases infectious complications and the patients receiving allogeneic units additional to the autologous transfusions in 53% of the cases.

Figure 5.1. Odds-ratios for infectious complications (autologous versus allogeneic group) per hospital. Hospitals are arranged in order of increasing odds-ratio. Significance of difference of odds-ratios; p=0.97. Common odds-ratio equals 1.2 (95% confidence interval, 0.8 to 1.9; p=0.49).

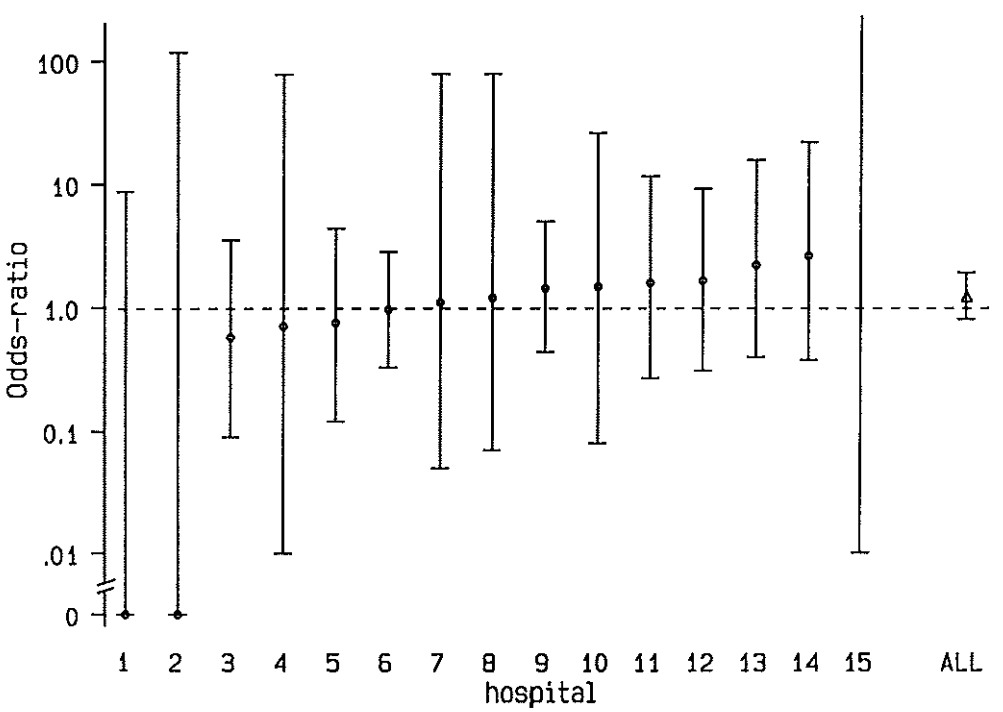

Table 5.1. Characteristics of the patients who were operated for colorectal cancer per randomized group.

	Allogeneic group (N = 234)		Autologous group (N = 236)	
Age (years)	68	(33-89)	66	(31-88)
Sex				
Male	131	(56%)	141	(60%)
Female	103	(44%)	95	(40%)
Operation				
No resection	2	(1%)	5	(2%)
Right hemicolectomy	30	(13%)	23	(10%)
Transverse colectomy	2	(1%)	5	(2%)
Left hemicolectomy	15	(6%)	16	(7%)
Sigmoid resection	45	(19%)	50	(21%)
Anterior resection	92	(39%)	87	(37%)
Abdominoperineal resection	47	(20%)	50	(21%)
Total colectomy	1	(1%)	-	-
Dukes' classification				
A	53	(23%)	55	(23%)
B	85	(36%)	80	(34%)
C	78	(33%)	72	(31%)
D	18	(8%)	29	(12%)
Histological differentiation				
Well	35	(15%)	33	(14%)
Moderate	169	(73%)	172	(74%)
Poor	28	(12%)	29	(12%)
Adjacent organ fixation	20	(9%)	26	(11%)
Tumor size (cm)	4.3	(0.5-13.0)	4.0	(0.8-15.0)
Hemoglobin concentration at base-line (g/dl)	14.5	(10.5-18.0)	14.4	(11.1-18.5)
Blood loss (ml)	775	(100-11,500)	750	(100-6,500)

Continuous data are presented as medians with ranges in parentheses and categorical data as numbers of patients with percentages in parentheses.

Table 5.2. Postoperative infectious complications after colorectal surgery.

	Allogeneic group (N = 234)		Autologous group (N = 236)	
Infections	58	(25%)	64	(27%)
Wound	14	(6%)	22	(9%)
Intra-abdominal	12	(5%)	18	(8%)
Urinary tract	19	(8%)	15	(6%)
Pneumonia	8	(3%)	6	(3%)
Other	5	(2%)	3	(1%)

Table 5.3. Univariate analysis of various risk factors of infectious complications.

	Total	Infections	p value	(trend)
Randomization				
Allogeneic group	234	58 (25%)	0.64	
Autologous group	236	64 (27%)		
Age (years)				
≤ 60	129	20 (16%)	0.004	(0.027)
61 - 70	172	55 (32%)		
> 70	169	47 (28%)		
Sex				
Male	272	69 (25%)	0.81	
Female	198	53 (27%)		
Operation				
Intra-abdominal	202	39 (19%)	0.006	
Rectal involvement	268	83 (31%)		
Dukes' classification				
A	108	33 (31%)	0.64	(0.48)
B	165	39 (24%)		
C	150	38 (25%)		
D	47	12 (26%)		
Adjacent organ fixation				
No	424	106 (25%)	0.21	
Yes	46	16 (35%)		
Tumor size (cm)				
≤ 3	108	27 (25%)	0.89	(0.68)
3 - 5	161	40 (25%)		
≥ 5	186	50 (27%)		
Base-line hemoglobin level (g/dl)				
≤ 13.7	171	41 (24%)	0.68	(0.39)
13.8 - 15.3	182	49 (27%)		
> 15.3	98	28 (29%)		
Blood loss (ml)				
≤ 500	154	31 (20%)	0.025	(0.010)
501 - 1000	138	31 (22%)		
> 1000	159	52 (33%)		
Plasma transfusions				
None	341	78 (23%)	0.038	
Allogeneic	57	21 (37%)		
Autologous	72	23 (32%)		
Blood transfusions				
None	159	33 (21%)	< 0.0001	
Allogeneic, 1 or 2 units	88	17 (19%)		
Autologous, 1 or 2 units	112	21 (19%)		
Allogeneic, > 2 units	62	25 (40%)		
Autologous and allogeneic	49	26 (53%)		

Note: Due to some missing data the total number of patients does not always equal 470.

In the allogeneic group 21 of the 101 patients who did not receive transfusions (21%) had infectious complications. The same percentage (12 of the 58 patients) was obtained in the patients who did not receive transfusions in the autologous group.
In Table 5.4 the risk of infectious complications is shown for the different groups of transfusions when taking into account the tumor location and the age of the patient. It was found that the group of patients receiving one or two allogeneic transfusions and the group receiving one or two autologous transfusions had a comparable risk of infectious complications as those patients who did not receive any transfusion. However, the patients who needed more than two units of blood had an increased risk of infections. This was the case in the allogeneic group (more than two units) and in the autologous group when additional allogeneic transfusions were given. Therefore more than two transfusions increased the risk of infectious complications. There was no significant difference whether the first two transfusions were of the allogeneic or autologous type. Plasma transfusion and blood loss, which were significantly related to infections when considered univariately, were no statistically significant additional influencing factors regarding the incidence of infectious complications in this regression analysis.

Table 5.4. Logistic regression analysis of various factors affecting the risk of infectious complications.

	Infections	Odds-ratio	95% C.I.	p value
Blood transfusions				
None	21%	1	-	-
Allogeneic, 1 or 2 units	19%	0.7	0.4 - 1.4	0.35
Autologous, 1 or 2 units	19%	0.8	0.4 - 1.4	0.42
Allogeneic, > 2 units	40%	2.0*	1.0 - 3.9	< 0.001
Autologous and allogeneic	53%	3.6*	1.8 - 7.3	0.047
Tumor location				
Intra-abdominal	19%	1	-	-
Rectal involvement	31%	1.7	1.0 - 2.7	0.036
Age (years)				
≤ 60	16%	1	-	-
61 - 70	32%	2.6	1.4 - 4.7	0.002
> 70	28%	2.1	1.2 - 3.9	0.015

* Not significantly different from each other (2.0 versus 3.6; p=0.14).

Discussion

This study shows that there is no beneficial effect of using autologous blood instead of allogeneic blood to reduce postoperative infectious complications in colorectal cancer patients. Heiss et al.[11] reported a randomized study similar to the present study, and

found that patients who were randomized to the autologous group had less infections. However, this study included only a total of 120 patients, and their finding was of marginal statistical significance. Also, they did not find a significant difference when the transfused patients of both randomized groups were compared.

We have previously shown that the donation of two units of blood had a significant effect on the immunological status of the patient.[12] However, the finding that the rate of infections of the patients who did not receive transfusions in both randomized groups were the same (21%), suggests that these changes do not affect the rate of infectious complications.

The other studies comparing the effect of allogeneic and autologous blood transfusions were uncontrolled.[13] Mezrow et al.[14] described a group of patients who underwent various surgical procedures, including a few colorectal cancer patients, and found that the infectious complication rate in the autologous recipients was reduced as compared to allogeneic recipients. However, the patients receiving autologous blood had shorter procedures, less blood loss, and needed fewer transfusions. Therefore, their findings were probably biased by imbalances between patients who underwent predeposit autologous donation and patients who did not. The study by Murphy et al.[15] found an advantage of autologous transfusions in patients who underwent total hip replacement. However, the patients receiving autologous blood were mainly operated by one surgeon and the patients transfused with allogeneic blood were operated by another surgeon. It is known from the study by McArdle and Hole[16] that infectious complications are surgeon related and therefore we believe that their conclusion that autologous transfusion decreased the rate of infectious complications is questionable. Fernandez et al.[17] also reported on orthopedic operations and infectious complications in relation to transfusions and failed to find any beneficial effect of autologous blood as compared to allogeneic blood. It was found that the only transfusion related factor affecting the incidence of infections was transfusion of whole blood.

The patients in these studies had to undergo procedures for which the operation could be postponed for a long time. For example, collection of autologous blood in orthopedic patients can take several months. Therefore additional allogeneic transfusions are less often needed than in colorectal cancer patients in which long postponement of the operation is not acceptable. Another difference is the rate of infectious complications. In orthopedic procedures infections were found in less than 10% of the cases whereas in colorectal surgery infectious complications occur in about 25% of the patients. The incidence of infections in our study are comparable to the other studies investigating the relationship between blood transfusions and infections after abdominal surgery.[18]

In contrast to the aforementioned studies our trial had a multicenter design which could have influenced the outcome. To investigate a possible diluting effect due to interhospital variance we calculated the odds-ratios for infections per hospital separately. None of the separate odds-ratios reached significance, neither did the odds-ratios differ significantly

from each other. Therefore there was no negative effect of multicentricity, whereas the advantage of such a design was the recruitment of a large amount of patients, which is necessary for proper conclusions on infectious complications.

It has been postulated that the immunologic changes induced by blood transfusions are a result of the transfusion of white cells.[19] Jensen et al.[20] reported a decreased incidence of wound infections after leukocyte depletion as compared to patients transfused with whole blood. However, no differences were found for the overall rate of infectious complications. A larger study by Houbiers et al.[21] compared filtered blood with buffy-coat depleted packed red cells, which is the same blood product as we used in our study. They found no significant difference between the study arms for the overall rate of infections nor the rate of wound infections. However, it was found that transfused patients had a significantly higher rate of infections than the patients who did not undergo transfusion. In regression analysis Houbiers et al. found, that the only influencing factors were transfusion of red cells and tumor location. In agreement with this our study showed that blood transfusion, the tumor location and the age of the patients did affect the risk of infections significantly. Because randomization was not a risk factor of infections we conclude that a predeposit autologous blood donation program does not reduce the incidence of infectious complications after colorectal cancer surgery.[22] There is no advantage of transfusing autologous blood for reducing postoperative bacterial infections. An important risk factor for infection is transfusion of more than two units of blood. An explanation for this finding could be that the immunologic changes induced by allogeneic transfusions are also generated by the transfusion of autologous blood. So far no such immunomodulatory effects of autologous blood have been demonstrated.[23,24,25] Therefore, the most likely explanation for our findings is that not the blood transfusions themselves but rather the circumstances necessitating them are the real factors influencing the risk of infectious complications. This is consistent with our earlier conclusion about the association between blood transfusions and colorectal cancer prognosis.[26] Of course blood transfusion is highly related to blood loss and surgical extent. It is known from animal and clinical studies that surgical trauma and blood loss induce immunosuppression.[12,27,28] It is therefore advisable to restrict these two factors to a minimum, thereby minimizing the need for transfusions. In colorectal cancer surgery this will be of benefit for the oncologic prognosis and the rate of infectious complications.

References

1. Tartter PI. Blood transfusion and infectious complications following colorectal cancer surgery. Br J Surg 1988; 75:789-92.
2. Wobbes Th, Bemelmans BLH, Kuypers JHC, Beerthuizen GIJM, Theeuwes AGM. Risk of postoperative septic complications after abdominal surgical treatment in relation to perioperative blood transfusion. Surg Gynecol Obstet 1990; 171:59-62.
3. Blumberg N, Heal JM. Transfusion and host defenses against cancer recurrence and infection. Transfusion 1989; 29:236-45.
4. Alexander JW. Transfusion-induced immunomodulation and infection. Transfusion 1991; 31:195-6.
5. Blumberg N, Heal JM. Transfusion-induced immunomodulation and its possible role in cancer recurrence and perioperative bacterial infection. Yale J Biol Med 1990; 63:429-33.
6. Pinto V, Baldonedo R, Nicolas C, Barez A, Perez A, Aza J. Relationship of transfusion and infectious complications after gastric carcinoma operations. Transfusion 1991; 31:114-8.
7. Busch ORC, Hop WCJ, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 328:1372-6.
8. Council on Scientific Affairs. Autologous blood transfusion. JAMA 1986; 256:2378-80.
9. Cox DR. The analysis of binary data. 1970 Methuen London.
10. Epidemiological Graphics, Estimation, and Testing package (EGRET), version 0.26.6, 1991, Software package, SERC Co. Seattle, USA.
11. Heiss MM, Mempel W, Jauch KW, et al. Beneficial effect of autologous blood transfusion on infectious complications after colorectal cancer surgery. Lancet 1993; 342:1328-33.
12. Marquet RL, Hoynck van Papendrecht MA, Busch OR, Jeekel J. Blood donation leads to decrease in natural killer cell activity: a study in normal blood donors and cancer patients. Transfusion 1993; 33: 368-373.
13. Triulzi DJ, Vanek K, Ryan DH, Blumberg N. A clinical and immunologic study of blood transfusion and postoperative bacterial infection in spinal surgery. Transfusion 1992; 32:517-24.
14. Mezrow CK, Bergstein I, Tartter PI. Postoperative infections following autologous and homologous blood transfusions. Transfusion 1992; 32:27-30.
15. Murphy P, Heal JM, Blumberg N. Infection or suspected infection after hip replacement surgery with autologous or homologous blood transfusions. Transfusion 1991; 31:212-7.
16. McArdle CS, Hole D. Impact of variability among surgeons on postoperative morbidity and mortality and ultimate survival. Br Med J 1991; 302:1501-5.
17. Fernandez MC, Gottlieb M, Menitove JE. Blood transfusion and postoperative infection in orthopedic patients. Transfusion 1992; 32:318-22.
18. Tartter PI, Quintero BA, Barron DM. Perioperative blood transfusion associated with infectious complications after colorectal operations. Am J Surg 1986; 152:479-82.
19. Persijn GG, Cohen B, Lansbergen Q, van Rood JJ. Retrospective and prospective studies on the effect of blood transfusions in renal transplantation in the Netherlands. Transplantation 1979; 28:396-401.
20. Jensen LS, Andersen AJ, Christiansen PM, et al. Postoperative infections and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. Br J Surg 1992; 79:513-6.
21. Houbiers JGA, Brand A, van de Watering LMG, et al. Randomised controlled trial comparing transfusion of leucocyte-depleted or buffy-coat-depleted blood in surgery for colorectal cancer. Lancet 1994; 344:573-8.
22. Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Autologous blood and infections after colorectal surgery. Lancet 1994; 343:668-9.
23. Waymack JP, Robb E, Alexander JW. Effect of transfusion on immune function in a traumatized animal model. II. Effect on mortality rate following septic challenge. Arch Surg 1987; 122:935-9.
24. Fischer E, Lenhard V, Seifert P, Kluge A, Johannsen R. Blood transfusion-induced suppression of cellular immunity in man. Hum Immunol 1980; 3:187-94.
25. Singh SK, Marquet RL, de Bruin RWF, Hop WCJ, Westbroek DL, Jeekel J. Consequences of blood loss on growth of artificial metastases. Br J Surg 1988; 75:377-9.

26. Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Blood transfusions and local tumor recurrence in colorectal cancer: evidence of a noncausal relationship. Ann Surg 1994; 220:791-7.
27. Stephan RN, Kupper TS, Geha AS, Baue AE, Chaundry IH. Hemorrhage without tissue trauma produces immunosuppression and enhanced susceptibility to sepsis. Arch Surg 1987; 122:62-8.
28. Guillou PJ. Biological variation in the development of sepsis after surgery or trauma. Lancet 1993; 342:217-20.

CHAPTER 6

BLOOD DONATION LEADS TO A DECREASE IN NATURAL KILLER CELL ACTIVITY: A STUDY IN NORMAL BLOOD DONORS AND CANCER PATIENTS

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Published in:
Transfusion 1993; 33: 368-373.

Summary

Transfusion-induced immunosuppression has long been known to be beneficial for organ transplantation patients, but recent retrospective studies suggest that blood transfusions may be detrimental for patients with cancer. If autologous blood is used to avoid immunosuppression, the assumption is that the procedure, involving blood donation, is immunologically neutral. In the present study, this assumption was evaluated by monitoring 33 normal blood donors and 16 colorectal cancer patients before and after donation of 1 (500 ml) and 2 units of blood, respectively. The cancer patients belonged to the autologous arm of a randomized trial in which the effects of allogeneic versus autologous blood on cancer prognosis were studied. The patients donated 2 units of blood with an interval of 3 to 4 days between donations. Flow cytometric analysis revealed that blood donation by normal donors and cancer patients had no effect on the proportion of B, T, and natural killer (NK) cells. Only the total number of lymphocytes was significantly decreased in the normal donors on Day 12 after donation. Blood donation had no significant effect on T cell function assessed by phytohemagglutinin stimulation in normal donors or in cancer patients donating 2 units of blood. A significant depression of NK cell function (88% and 74% of predonation levels) was observed in normal donors on Days 2 and 5 after donation; on Day 12, the activity was again normal. Colorectal cancer patients had a significantly depressed NK cell activity (54% of predonation activity) on Day 12 after the first donation. Before donation, the NK cell activities of blood donors and cancer patients were similar (45.6 ± 4.3% and 41.4 ± 3.6%, respectively, at the 50:1 ratio). Before donation, the number of NK cells correlated significantly with NK cell activity, but, after donation, such a correlation was lacking. This study indicates that blood donation, especially the donation of 2 units, leads to a depressed NK cell activity. If confirmed in a larger study, this finding may have important implications for cancer patients undergoing surgery.

Introduction

Autologous blood is becoming increasingly popular as an alternative for allogeneic blood. This change in blood transfusion policy is based on the awareness that allogeneic transfusions involve the risk of infection and induction of immunosuppression.[1,2] Transfusion-induced immunosuppression has long been known to be beneficial for organ transplantation patients, but recent investigations suggest that it may be harmful for patients with cancer.[3,4] Various retrospective studies have identified allogeneic blood transfusion as an independent risk factor for recurrence in and survival of cancer patients.[5] However, in other studies,[6,7] such a correlation could not be demonstrated. Although this putative negative effect of blood transfusion has not yet been confirmed by prospectively randomized clinical studies, it has stimulated considerably the use of preoperatively deposited autologous blood. When autologous blood is used to avoid immunosuppression, an important assumption is that the donation and transfusion of this blood are immunologically neutral. This assumption may be wrong, since it is known from animal studies that hemorrhage can lead to a marked and long-lasting depression of both specific and nonspecific immunity.[8] It has also been demonstrated that long-term blood donation can lead to a depression in natural killer (NK) cell activity in the blood donors, without, however, leading to any adverse clinical manifestation.[9] Although these studies relate to rather extreme situations that do not occur during the preoperative deposit of autologous blood, they still are intriguing and warrant further investigation of the immunologic effects of simple blood donation. The question addressed in the current study therefore was whether the donation of 1 or 2 units of blood might lead to some form of immunosuppression. The investigations were performed in the period immediately following blood donation and employed normal blood donors and colorectal cancer patients participating in a preoperatively deposited autologous blood transfusion program.[10]

Materials and methods

Normal donors and cancer patients

Thirty-three blood donors and 16 colorectal cancer patients were included in the study. The normal blood donors were recruited from volunteer donors at the Red Cross Blood Bank in Rotterdam, The Netherlands. They were all more than 40 years old and had been donating blood for more than two years, at a frequency of twice per year. After they gave informed consent, 40 ml of blood for immunologic testing was taken before and after the donation of 1 unit (500 ml) of blood. The first group of 18 donors (11 men, 7 women) was tested immediately before donation and 2 and 5 days after donation. Because we found that the NK cell activity in this group was still abnormal on Day 5, we tested a

second group of 15 blood donors (5 men, 8 women) to see whether NK cell activity would still be low 12 days after donation. The NK cell activity in this group was measured before donation and on Days 5 and 12.

The 16 colorectal cancer patients were participants in a multicenter trial on the effect of blood transfusions on cancer recurrence and survival.[10] In this trial, patients were randomly assigned to receive preoperatively deposited autologous blood or allogeneic blood (buffy-coat depleted). The patients included in the current immunologic study were randomly assigned to the autologous arm of the trial, and all were scheduled for potentially curative surgery at the University Hospital Dijkzigt (Rotterdam). There were seven men and nine women, ranging in age from 31 to 84 years. We did not include patients with a history of blood transfusion or those who had earlier cancer, familial polyposis, or ulcerative colitis. The patients fulfilled the criteria set for autologous blood donation by the American Association of Blood Banks.[11] The study was approved by the ethics committee of the hospital. The patients donated a total of 2 units of blood with an interval of 3 to 4 days between the two donations. Blood for immunologic monitoring was taken 2 days before the first donation and 8 to 9 days after the second donation (Day 12).

Flow cytometry

We analyzed subsets of lymphoid cells with a fluorescence-activated cell scanner (FACScan, Becton Dickinson, Mountain View, CA, USA) and used software (SimulSET, Becton Dickinson) for lymphocyte gating and determination of proportions of lymphocyte subsets. We mixed 100 µl of heparinized blood in separate tubes with 20 µl of the monoclonal antibodies listed in Table 6.1 for 30 minutes at room temperature. All monoclonal antibodies were purchased from Becton Dickinson. Red cells were lysed (FACS lysing solution, Becton Dickinson), and the remaining lymphoid cells were washed three times with phosphate-buffered saline. The first sample from a given subject was used to identify the proportions of lymphocytes, monocytes, and granulocytes. This was done by a combination of antigen expression (CD14 on monocytes, granulocytes, and macrophages) and light-scattering characteristics. Gates were set to exclude the lymphocytes, and this gate setting was used for all subsequent samples from that person. In the second sample, we used antibodies against keyhole-limpet hemocyanin (KLH) as control for aspecific binding. We calculated absolute numbers of the different subsets by multiplying the proportion of positive cells by the total number of lymphocytes.

Phytohemagglutinin stimulation assay

Peripheral blood mononuclear cells were isolated by gradient centrifugation using lymphocyte separation medium (Litton Bionetics, Chatsworth, CA). They were washed three times and resuspended in medium (RPMI-1640, Gibco Laboratories, Grand Island, NY) containing 10% fetal calf serum and 10^5 mol/l β-mercaptoethanol. Using round-bottom microtiter plates (Nunc, Roskilde, Denmark), we added 20 µl of phyto-

hemagglutinin (PHA; Wellcome, Dartford, UK) in different concentrations (6.25, 12.5, 25, 50, and 75 µg/ml) to triplicate cultures of 20 µl of lymphocyte suspension containing 7.5 x 10^6 cells per ml. Six hours before termination, each culture was labeled with 0.8 µCi of methyl-3-H-thymidine (specific activity, 2 Ci/mmol; Amersham, UK). The cultures were harvested with an automatic microtiter plate harvester (Automash, Dynatech, Baton Rouge, LA). We collected cells on fiberglass filters; after drying them, we placed the filters in vials, added scintillation fluid, and determined the uptake of methyl-3-H-thymidine in a liquid scintillation counter.

Table 6.1. Monoclonal antibodies used in flow cytometry.

Sample	Antigen	Antibody	Cellular distribution
1	CD14	Anti-leuM3-PE	Monocytes Granulocytes Macrophages
2	KLH	Ig-G2-PE	Control-aspecific
2	KLH	Ig-G1-FITC	binding
3	CD19	Anti-leu12-PE	B cells
3	CD3	Anti-leu4-FITC	T cells
4	CD4	Anti-leu3a-PE	T4 cells
4	CD8	Anti-leu2a-FITC	T8 cells
5	CD16	Anti-leu11-PE	NK cells Granulocytes Macrophages
5	CD8	Anti-leu2a-FITC	T8 cells
6	CD16	Anti-leu11-PE	NK cells
6	CD57	Anti-leu7-FITC	Granulocytes Macrophages NK cells
7	CD3	Anti-leu4-PE	T cells
7	CD57	Anti-leu7-FITC	NK cells

PE: Phycoerythrin, red fluorescence
FITC: Fluorocein isothiocyanate, green fluorescence
KLH: Keyhole limpet hemocyanin
T4: T helper/inducer cells
T8: T cytotoxic/suppressor cells

Natural killer (NK) cell assay

For the NK cell assay, we used the human erythroleukemic cells, K562, which are the prototype of NK-sensitive targets. NK cell activity was measured in a 4-hour assay using

gradient-separated lymphocytes and ^{51}Cr-labeled K562 target cells. We performed the assay in triplicate in round-bottom microtiter plates (Nunc) by adding lymphocytes to 1 x 10^4 target cells at ratios of 6.25:1, 12.5:1, 25:1, and 50:1. The plates were centrifuged for 3 minutes at 150 x *g* and then incubated for 4 hours at 37°C in a humidified 5% CO_2 incubator. To harvest the culture, we centrifuged plates for 3 minutes at 150 x *g* and removed the supernatants using a collection system (Skatron, Lier, Norway).[12] The release of label was determined by counting radioactivity in a gamma counter (counts per minute = cpm). We assessed spontaneous release (SR) in target cell cultures without lymphocytes and determined the maximal release (MR) by adding 10% cetavlon detergent. The percentage of specific lysis was calculated according to the following formula:

$$\frac{\text{mean cpm experimental - mean cpm SR}}{\text{mean cpm MR - mean cpm SR}} \times 100\%$$

NK cell activities are presented for the 50:1 lymphocyte-to-target cell ratio.

Statistical analysis

The significance of differences was evaluated by using the Mann-Whitney test and the paired Wilcoxon test. A p value <0.05 was considered statistically significant.

Results

Effect of blood donation on lymphocyte subsets

We performed subset analysis in the second group of 15 normal blood donors and in the group of colorectal cancer patients. The normal volunteers donated 1 unit of blood, and samples for monitoring were taken before donation and on Days 5 and 12 after donation. The cancer patients donated 2 units of blood with an interval of 3 to 4 days between donations; monitoring was done before the first donation and 12 days after. In the normal blood donors, the number of white cells remained stable after donation. The mean percentage of lymphocytes dropped from 32.6% before donation and 33.3% on Day 5 to 25.4% on Day 12 ($p=0.02$) (Table 6.2). As a consequence, the absolute number of circulating lymphocytes on Day 12 was less than before donation ($p=0.01$). The proportions of B, T, and NK cells did not change, nor did the CD4:CD8 ratio, but the absolute numbers were significantly lower than before donation (Figure 6.1). In the colorectal cancer patients the number of white cells and the percentage of lymphocytes did not change after blood donation. Nor did the proportion and number of B, T, and NK cells change significantly (Table 6.2 and Figure 6.1).

Figure 6.1. Effect of blood donation by normal donors and colorectal cancer patients on number (x 10^6/l) of lymphocytic subpopulations (see Table 6.1).

The upper part gives the results of normal blood donors before and after donation of 1 unit of blood; the lower part represents the results obtained in colorectal cancer patients before and after donation of 2 units of blood. (* denotes p value <0.05).

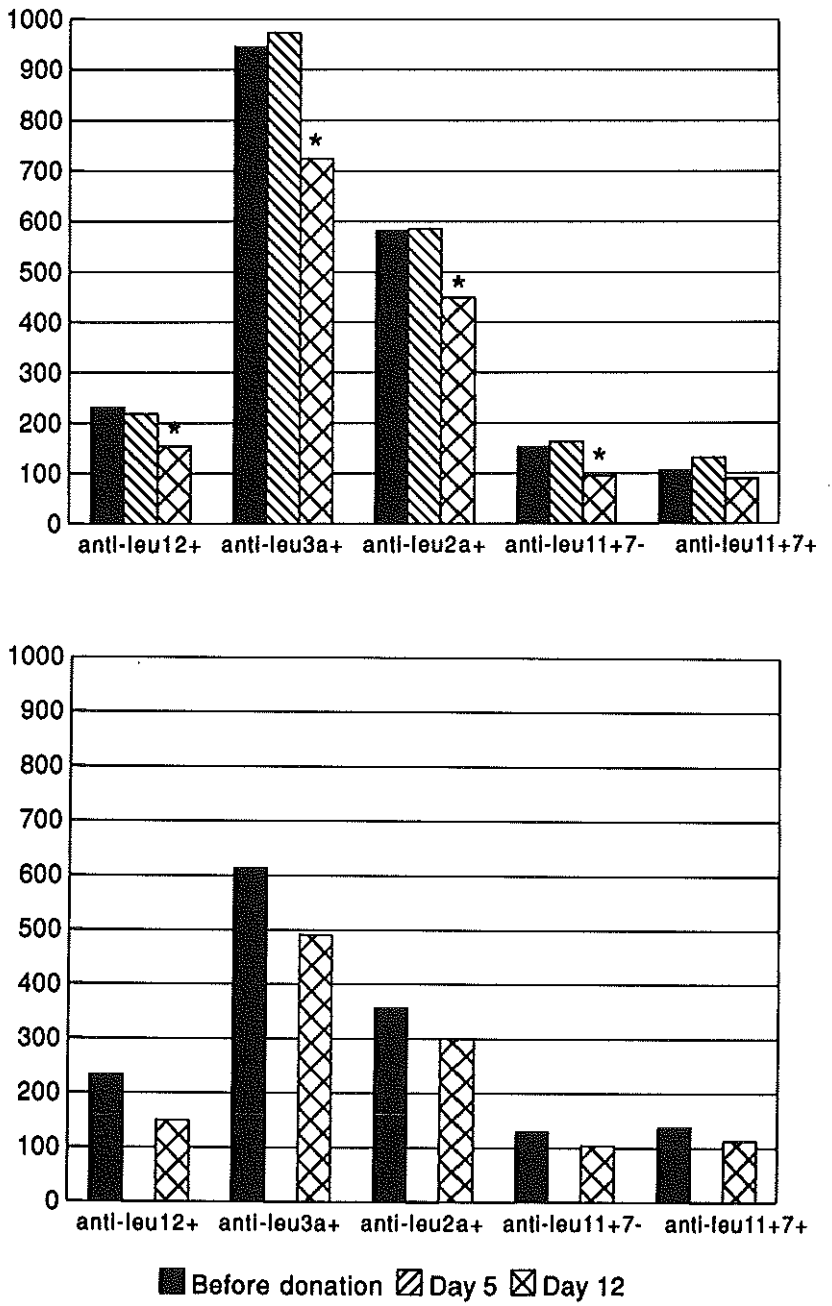


Table 6.2. Effect of blood donation on the proportion of lymphocytes and lymphocyte subsets.

	Lymphocytes	B cells	T cells	NK cells
Blood donors				
Day 0	32.6 %	11.3 %	74.6 %	12.9 %
Day 5	33.3 %	10.6 %	75.1 %	13.5 %
Day 12	25.4 %*	10.2 %	76.1 %	12.5 %
Colorectal cancer patients				
Day -2	23.2 %	15.8 %	65.2 %	18.7 %
Day 12	19.8 %	12.2 %	69.5 %	17.8 %

* Significantly (p=0.02) different as compared with Day 0 level.

Effect of blood donation on T lymphocyte function

Donation of 1 unit of blood by the first group of 18 voluntary blood donors did not result in a significant change in PHA response immediately after donation or on Days 2 and 5. Donation of 2 units of blood by colorectal cancer patients led to a mean decrease of 30% in PHA response on Day 12; however, this decrease did not reach significance (Table 6.3).

Table 6.3. Effect of blood donation on PHA response.*

	PHA response (mean ± SEM)	Range
Blood donors		
Day 0	122.8 ± 9.5	19 - 190
Day 2	126.4 ± 9.3	42 - 186
Day 5	116.8 ± 7.3	73 - 177
Colorectal cancer patients		
Day -2	70.1 ± 11.9	25 - 130
Day 12	49.0 ± 13.1	9 - 112

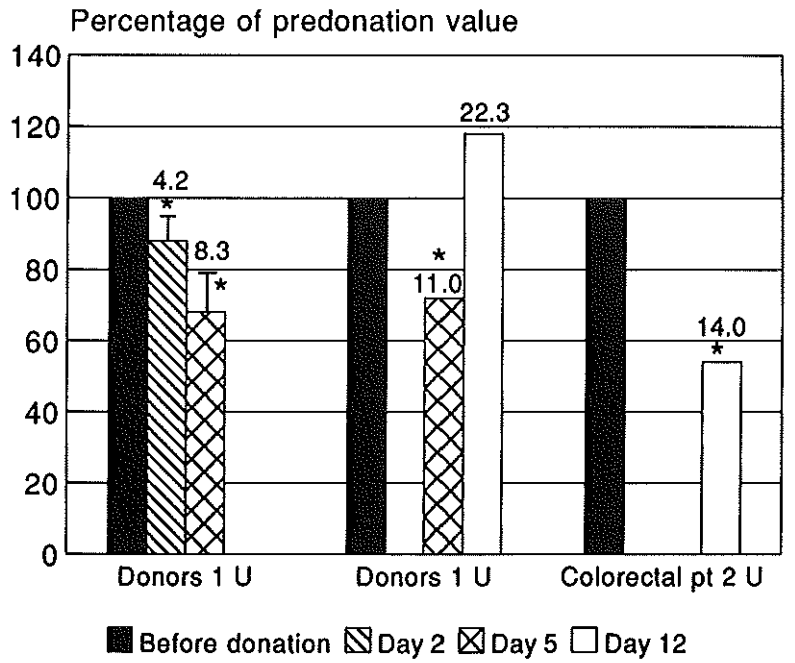
* The results are given in counts x 10^3 per minute.

Effect of blood donation on NK cell activity

In the first group of blood donors, the NK cell activity, relative to predonation activity, was 104% immediately after donation, 88% on Day 2 (p=0.02), and 68% on Day 5 (p<0.02). We observed a similar depression (74% of predonation activity) on Day 5 in the second group of donors, whereas on Day 12, the NK cell activity was again normal (117%) (Figure 6.2).

Figure 6.2. Effect of blood donation on NK cell activity (± SD) in healthy blood donors donating 1 unit of blood and in colorectal cancer patients donating 2 units of blood.

NK cell activity is given as percentage of predonation value. (* denotes p value <0.05).



Colorectal cancer patients were tested before and 12 days after the first donation, or 9 days after the second donation. On Day 12, the NK cell activity was only 54% of predonation activity (p=0.002) (Figure 6.2). There was no difference in predonation NK cell activity in blood donors and cancer patients. The specific release at the 50:1 lymphocyte-to-target cell ratio was 41.4 ± 3.6% (mean ± SEM) in colorectal cancer patients and 45.6 ± 4.3% in blood donors. Before donation, there was a significant correlation between NK cell activity and the proportion of NK cells analyzed by flow cytometry (p=0.016). The correlation was 0.61 with regard to Leu-11^+7^- cells and 0.71 for Leu-11^+7^+ cells. There was no significant correlation between NK cell activity and the proportion of Leu-11^-7^+ cells. These cells appeared to be positive for CD3 markers and thus probably were T cells. The decreased NK cell activity observed in normal blood donors on Day 5 and in colorectal cancer patients on Day 12 no longer correlated significantly with the total proportion of NK cells. The correlation was 0.44 on Day 5 and 0.35 on Day 12.

Chapter 6

Discussion

The present observations demonstrate a significant decrease in NK cell activity in voluntary blood donors and cancer patients after the donation of 1 or 2 units of blood, respectively. Donation of 1 unit of blood resulted in a decreased NK cell activity lasting for at least 5 days. In cancer patients who donated 2 units of blood, this decrease was still demonstrable after 12 days, which suggests that the duration of NK cell depression may be related to the amount of blood taken. Alternatively, it is conceivable that blood donation has a more profound impact on NK cell function in cancer patients than in healthy blood donors. Decreased NK cell activity in relation to blood donation was observed earlier by Lasek et al.[9] However, those investigators focused not on the phase immediately following blood donation but on the long-term effect of frequent donation. Patients were monitored at least 3 months after the last donation. It was found that the NK cell activity in frequent blood donors was significantly decreased as compared to that in age-matched controls. The magnitude of the decrease appeared to be related to the total amount of blood donated, with the lowest values seen in active long-term donors. In seeming contrast to these findings are the observations by Lewis et al.,[13] who found that long-term blood donation had no effect on the percentage of NK cells in peripheral blood. However, NK cell activity was not measured, though this may be more important than monitoring of NK cell numbers. Our findings indicate that there indeed may exist a discrepancy between the proportion of NK cells and NK cell activity. Although the proportion of NK cells remained unaffected after blood donation, we still observed that the NK cell activity dropped significantly. Prolonged impairment of NK cell activity following simple hemorrhage without tissue trauma has been demonstrated in mice by Stephan et al.[14] These investigators drew blood from mice until they reached a mean blood pressure of 35 mmHg, which was maintained for 1 hour, after which the animals were resuscitated. Up to 6 days after hemorrhage, the NK cell activity of spleen cells was profoundly depressed. The same group of investigators demonstrated that also T cell related functions like PHA response, mixed lymphocyte reaction, and interleukin-2 production also were severely depressed following hemorrhage.[15,16] In our study, we did not observe a significant change in T cell function at any time after blood donation. The only alteration observed was that, at 12 days after the donation of 1 unit of blood, the absolute number of T cells was decreased.

The depression of NK cell activity by blood donation is the major finding of our study may be caused by several mechanisms, as proposed earlier by others.[9] First, it is known that hemorrhage is a potent stimulator of corticosteroid and prostaglandin secretion. These mediators have been demonstrated to inhibit NK cell activity, and they may have been responsible for the decline in NK cell activity after blood donation. Second, it is possible that blood donation leads to a shifting of mature NK cells from the blood to the bone marrow and lymphoid organs, which leads to the recruitment in the blood of immature NK

cells with lower activity. This explanation is not likely to hold for our present findings, since it was found that, after donation, the absolute number and proportion of NK cells remained unchanged, whereas their activity decreased.

The important question remains whether the results emerging from our study bear any clinical relevance. NK cells are believed to play a role in the first-line defense against tumor growth and infection.[17,18] Therefore, a reduced NK cell activity may be detrimental for normal patients and even more so for patients with cancer. Evidence that NK cells are involved in tumor development and metastasis is mainly derived from tumor transplantation studies in rodents. Among the many studies performed on this subject, a crucial observation was that experimental tumor metastasis was enhanced in strains of mice with low NK cell activity but reduced in strains exhibiting high NK cell activity.[19,20] Furthermore, the selective elimination of NK cells by monoclonal antibodies has been reported to decrease the survival of animals injected with tumor cells.[21] In the clinical situation, the role of NK cells in growth and destruction of tumors is less clear. A correlation between low NK cell activity and tumor progression has been reported by many authors,[22] and, conversely, high NK cell activity at the site of the tumor has been found to correlate with tumor regression.[23] The strongest support for the notion that NK cells may be of significance in cancer is provided by studies in which the adoptive transfer of interleukin-2 activated NK cells was found to have some therapeutic effect.[24]

The role of NK cells with regard to susceptibility for infection seems less speculative. In murine models, it has been found that the depletion of NK cells resulted in a marked increase in death as a result of otherwise sublethal challenges with virus.[25] More important, a recent study performed in healthy volunteers demonstrated that subjects exhibiting a persistently low level of NK cell activity were more at risk for developing infectious disease than individuals with normal NK cell activity.[26] In addition, Siegal et al.[27] showed that opportunistic infections in acquired immune deficiency syndrome patients developed only if both T cell and NK cell activity were depressed.

It seems reasonable to assume that the short-term depression of about 30% of NK cell activity observed in our group of normal blood donors fell within the range of normal physiologic fluctuation of NK cell activity and has no clinical significance. It has been demonstrated earlier that the intra-individual day-to-day variation in NK cell activity using K562 targets is about 13%, and that the variance between subjects is 20%.[28] The importance of the depression in NK cell activity may be quite different for the cancer patients participating in the autologous blood transfusion trial. These patients had donated 2 units of blood and were still low in NK cell activity 12 days after the first donation. They were scheduled for major surgery on Day 14 and presumably were still low in NK cell activity at the time of operation. It is conceivable that, through this depression in NK cell activity, in conjunction with the immunosuppression evoked by surgery, these patients are more susceptible for the development of metastases and infection. We showed earlier

in an artificial metastasis model in rats[29] that the donation of blood, alone or in combination with surgery, leads to enhanced tumor growth. In addition, blood donation in this rat model led to a significant decrease of NK cell activity.[30] It has frequently been demonstrated that, during surgery for cancer, many tumor cells are released into the circulation; the homing of these cells may be facilitated by the low level of NK cell activity at this crucial moment.[31] Relevant in this respect is the finding by Tartter et al.[32] that colorectal cancer patients with low preoperative levels of NK cell activity were at increased risk for tumor recurrence. In addition, an impaired NK cell function has recently been linked to the occurrence of wound infections in patients undergoing elective colorectal surgery.[33]

It is obvious that our preliminary results on the effects of postdonation NK cell activity require confirmation in larger groups of patients before definite conclusions can be drawn or measures for intervention can be advocated.

References

1. de Waal LP, van Twuyver E. Blood transfusion and allograft survival: is mixed chimerism the solution for tolerance induction in clinical transplantation? Crit Rev Immunol 1991; 10:417-25.
2. Opelz G, Mickey MR, Terasaki PI. Identification of unresponsive kidney transplant recipients. Lancet 1972; 1:868-71.
3. Tartter PI, Burrows L, Papatestas AE, Lesnick G, Aufses AH Jr. Perioperative blood transfusion has prognostic significance for breast cancer. Surgery 1985; 97:225-30.
4. van Lawick van Pabst WP, Langenhorst BLAM, Mulder PGH, Marquet RL, Jeekel J. Effect of perioperative blood loss and perioperative blood transfusion on colorectal cancer survival. Eur J Cancer Clin Oncol 1988; 24:741-7.
5. Blumberg N, Heal JM. Transfusion and host defenses against cancer recurrence and infection. Transfusion 1989; 29:236-45.
6. Foster RS, Foster JC, Constanza MC. Blood transfusions and disease-free survival after surgery for breast cancer. Arch Surg 1984; 119:1138-40.
7. Nowak MM, Ponsky JL, Blood transfusion and disease-free survival in carcinoma of the breast. J Surg Oncol 1984; 27:124-30.
8. Chaudry IH, Ayala A, Ertel W, Stephan RN. Hemorrhage and resuscitation: immunological aspects. Am J Physiol 1990; 259:R663-78.
9. Lasek W, Plodziszewska M, Jakobisiak M. The effect of blood donation on natural killer activity in man. J Clin Lab Immunol 1987; 22:165-8.
10. Hoynck van Papendrecht MAW, Hop W, Langenhorst BLAM, Kothe FC, Marquet RL, Jeekel J. Feasibility of a predeposit autologous blood donation program in colorectal cancer: results from a randomized clinical study. Vox Sang 1992; 62:102-7.
11. Council on Scientific Affairs. Autologous blood transfusions. JAMA 1986; 256:2378-80.
12. Hirschberg H, Skare H, Thorsby E. Cell mediated lympholysis: CML. A microplate technique requiring few target cells and employing a new method of supernatant collection. J Immunol Methods 1977; 16:131-41.
13. Lewis SL, Kutvirt SG, Simon TL. Investigation of the effect of long-term whole blood donation on immunologic parameters. Transfusion 1992; 32:51-6.
14. Stephan RN, Poo WJ, Janeway CA, Zoghbi SS, Dean RE, Geha AS, Chaudry IH. Prolonged impairment of natural killer (NK) activity following simple hemorrhage. Circ Shock 1987; 21:312-3.
15. Stephan RN, Conrad PJ, Janeway CA, Geha AS, Baue AE, Chaudry IH. Decreased interleukin-2 production following simple hemorrhage. Surg Forum 1986; 37:73-5.
16. Stephan RN, Kupper TS, Geha AS, Baue AE, Chaudry IH. Hemorrhage without tissue trauma produces immunosuppression and enhances susceptibility to sepsis. Arch Surg 1987; 122:62-8.
17. Herberman RB, Ortaldo JR. Natural killer cells: their roles in defenses against disease. Science 1981; 214:24-30.
18. Oldham RK. Natural killer cells: history, relevance and clinical application. Nat Immun Cell Growth Regul 1990; 9:297-312.
19. Gorelik E, Herberman RB. Susceptibility of various strains of mice to urethane-induced lung tumors and depressed natural killer cell activity. J Natl Cancer Inst 1981; 66:1317-22.
20. Hanna N, Fidler IJ. Relationship between metastatic potential and resistance to natural killer cell-mediated cytotoxicity in three murine tumor systems. J Natl Cancer Inst 1981; 66:1183-90.
21. van den Brink MRM, Hunt LE, Hiserodt JC. In vivo treatment with monoclonal antibody 3.2.3. selectively eliminates natural killer cells in rats. J Exp Med 1990; 171:197-210.
22. Pross HF, Baines MG. Spontaneous human lymphocyte-mediated cytotoxicity against tumour target cells. I. The effect of malignant disease. Int J Cancer 1976; 18:593-604.
23. Lotzova E, Savary CA, Freedman RS, Bowen JM. Natural killer cell antitumor activity in patients with ovarian carcinoma: induction of cytotoxicity by viral oncolysates and interleukin-2. In: Rutledge FN, Freedman RS, Gershenson DM, eds. Annual Clinical Conference on Cancer. Austin: University of Texas Press, 1987; 29:123-36.
24. Rosenberg SA. The development of new immunotherapies for the treatment of cancer using interleukin-2. Ann Surg 1988; 218:121-35.

25. Bukowski JF, Woda BA, Habu S, Okumura K, Welsh RM. Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo. J Immunol 1983; 131:1531-8.
26. Levy SM, Herberman RB, Lee J, et al. Persistently low natural killer cell activity, age, and environmental stress as predictor of infectious morbidity. Nat Immun Cell Growth Reg 1991; 10:289-307.
27. Siegal FP, Lopez C, Fitzgerald P, et al. Opportunistic infections in acquired immune deficiency syndrome result from synergistic defects of both the natural and adaptive coponents of cellular immunity. J Clin Invest 1986; 78:115-23.
28. Porzsolt F, Gaus W, Heimpel H. The evaluation of serial measurements of the NK cell activity in man. Immunobiology 1983; 165:475-84.
29. Singh SK, Marquet RL de Bruin RW, Hop WCJ, Westbroek DL, Jeekel J. Consequences of blood loss on growth of artificial metastases. Br J Surg 1988; 75:377-9.
30. Hoynck van Papendrecht MAW, Busch ORC, Jeekel J, Marquet RL. The influence of blood loss on tumour growth: effect and mechanism in an experimental model. Neth J Surg 1991; 43:85-8.
31. Griffiths JD, McKinna JA, Rowbotham HD, Tsolakidis P, Salsbury AJ. Carcinoma of the colon and rectum: circulating malignant cells and five-year survival. Cancer 1973; 31:226-36.
32. Tartter PI, Steinberg B, Barron DM, Martinelli G. The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. Arch Surg 1987; 122:1264-8.
33. Jensen LS, Andersen AJ, Christiansen PM, et al. Postoperative infection and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. Br J Surg 1992; 79:513-6.

CHAPTER 7

THE EFFECT OF BLOOD DONATION ON PROGNOSIS AND INFECTIOUS COMPLICATIONS AFTER COLORECTAL CANCER SURGERY

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Submitted for publication.

Summary

Autologous blood transfusions are used to overcome the immunological consequences induced by allogeneic blood. However, predeposit autologous blood transfusion requires blood donation, which has been demonstrated by us to decrease natural killer cell activity in colorectal cancer patients. This study investigates the effects of blood donation on prognosis and infectious complications after colorectal cancer surgery. We analyzed a subgroup of patients (N=135) who had not required transfusions in a randomized trial comparing the effects of autologous versus allogeneic blood transfusions in colorectal cancer surgery. Patients in the autologous group had donated two units of blood preoperatively.

In the control group 22% (20/92) and in the donation group 16% (7/43) had infectious complications (p=0.61). Differences regarding the risk of infections were also not demonstrated using multivariate analysis.

The colorectal cancer-specific survival at 4 years of all patients was 83% in the control group and 72% in the donation group (p=0.30). The disease-free survival at 4 years of 121 patients who underwent curative surgery was 74% in the control group and 78% in the donation group (p=0.94). Multivariate analysis also did no reveal significant differences in prognosis between the groups.

We conclude that the donation of two units of blood for autologous transfusion in colorectal cancer patients did neither affect the incidence of infectious complications, nor affect the prognosis of those patients.

Introduction

In the last decades autologous blood transfusions have become more popular because doctors and patients fear the risks of allogeneic transfusions. Especially the risk of transmission of viral infections, but also the induction of immunosuppression by allogeneic blood transfusions may be harmful for operated patients. Although recently prospective studies have given more insight whether transfusion-induced immunosuppression has a negative effect on cancer prognosis, this topic is still not fully explained.[1] However, it is known that preoperative autologous blood donation is a safe procedure and therefore it is one of the best alternatives for third party (allogeneic) blood transfusions.
In a multicenter randomized clinical study in colorectal cancer patients, we investigated the effects of autologous blood transfusions as compared to standard allogeneic transfusions. We came to the conclusion that a detrimental effect of allogeneic transfusions on cancer prognosis, whenever it exists, can not be overcome by the use of a predeposit autologous blood transfusion program. Obviously, the use of autologous blood transfusions requires the donation of blood. Blood donation in healthy donors can reveal changes in immunologic parameters and we have found also that blood donation leads to a significant decrease in natural killer cell activity in colorectal cancer patients.[2,3] The present study was performed to investigate whether blood donation prior to surgery for colorectal cancer had any effect on prognosis and on the incidence of postoperative infectious complications. For this purpose we analyzed a subgroup of patients who participated in the above mentioned randomized study. To eliminate the putative effects of blood transfusions we excluded the patients who received any type of transfusion from the present analyses.

Materials and methods

The evaluated patients participated in a randomized multicenter trial to investigate the effect of autologous blood transfusions on prognosis of colorectal cancer as compared to standard allogeneic transfusions. The design of this trial has been described in detail elsewhere.[4] This multicenter study was performed in 15 hospitals and was approved by the ethics committees of all the participating centers. After written informed consent had been obtained, eligible patients were randomly assigned to either the allogeneic group or the autologous group. The enrollment of the patients took place from August 1986 to November 1991. All patients who were scheduled for a curative resection of colorectal malignancy were eligible for the study, if they fulfilled the criteria set for autologous blood donation by the American Association of Blood Banks.[5] These criteria required the absence of severe cardiovascular or respiratory disease, no history of epilepsy after infancy, and a hemoglobin concentration above 11.3 g/dl (7 mmol/l). In addition, the patients had no history of blood transfusion during the last three months before randomization.

The patients who were assigned to the autologous group were required to donate blood twice. The minimum interval between the two donations was 72 hours, and the second donation had to occur not later than five days before surgery. At each donation, 450 ml of blood was obtained by standard procedures. The patients were treated with oral iron supplementation, ferrous fumarate 3 times daily 200 mg, immediately after randomization. The collected blood was separated into packed red blood cells (without buffy coat) and fresh-frozen plasma, except at one hospital, where autologous blood was given in transfusion as whole blood. Packed cells were given if blood loss exceeded 500 ml or the hemoglobin level dropped below 10.5 g/dl. Fresh-frozen plasma was transfused when indicated.

Antibiotic prophylaxis and bowel irrigation were given routinely. After standard surgical procedures the tumors were staged according to the Turnbull[6] modification of the Dukes' classification. The patients underwent a standard follow-up program, and when indicated histologic or cytologic evidence of recurrent disease was obtained.

In the present study, which was performed to explore the effect of blood donation in patients who were operated for colorectal cancer, all transfused patients and the patients in the autologous group who did not donate blood were left out of the analyses. Consequently, 135 of the 475 randomized patients remained for the present analysis. Of these patients the one in the autologous group were exposed to blood donation and called the donation group, whereas the patients in the allogeneic group were called the control group.

Colorectal cancer-specific survival and disease-free survival curves were calculated according to the Kaplan-Meier method,[7] and the log rank test was used to compare these curves. Multivariate analyses of survival data were performed by Cox regression.[8] Percentages were compared by the chi-square test. Logistic regression[9] was used to investigate various factors simultaneously regarding the incidence of infectious complications. Two-sided p values ≤ 0.05 were considered statistically significant.

Results

Of the 475 patients who participated in the above mentioned randomized multicenter trial, 135 patients remain for this study. Of these, 92 patients were in the control group and 43 patients in the donation group. All the 43 patients in the donation group donated two units of blood prior to surgery. The characteristics of the patients were not significantly different between the two groups (Table 7.1). The discrepancy between both groups regarding the number of patients can be explained by the fact that patients in the autologous group more often required blood transfusions as a result of the hemoglobin decrease by blood donation.

Table 7.1. Characteristics of the untransfused patients per group.

	Control group (N = 92)	Donation group (N = 43)
Age		
≤ 60 years	26 (28%)	16 (37%)
> 60 & ≤ 70 years	35 (38%)	16 (37%)
> 70 years	31 (34%)	11 (27%)
Sex		
Male	51 (55%)	26 (61%)
Female	41 (45%)	17 (40%)
Tumor location		
Intra-abdominal	56 (61%)	28 (65%)
Rectal involvement	36 (39%)	15 (35%)
Dukes' classification		
A	25 (27%)	11 (26%)
B	32 (35%)	10 (23%)
C	28 (30%)	15 (35%)
D	7 (8%)	7 (16%)
Histological differentiation		
Well	13 (14%)	7 (16%)
Moderate	69 (75%)	30 (70%)
Poor	10 (11%)	6 (14%)
Adjacent organ fixation		
No	86 (94%)	41 (95%)
Yes	6 (6%)	2 (5%)

Table 7.2. Logistic regression of various factors regarding the risk of infectious complications of untransfused patients (N=135).

Factor	No. of patients infected/total (%)	Odds ratio	95% confidence interval	p value
Tumor location				
Intra-abdominal	14/84 (17%)	1		
Rectal involvement	13/51 (26%)	1.9	0.8 - 4.7	0.16
Age				
≤ 60 years	3/42 (7%)	1		
> 60 & ≤ 70 years	11/51 (22%)	4.0	1.0 - 16.0	0.046
> 70 years	13/42 (31%)	5.9	1.5 - 23.1	0.01
Study group				
Control group	20/92 (22%)	1		
Donation group	7/43 (16%)	0.8	0.3 - 2.1	0.65

In the control group 20 patients (22%) had postoperative infectious complications as compared with 7 patients (16%) in the donation group (p=0.61). In logistic regression analysis, including also tumor location and age of the patient, the factor study group was not an influencing factor for the risk of infectious complications (Table 7.2). Only the age of the patient was a significant risk factor of infections.
During the study, 12 patients in the control group and 9 patients in the donation group died of colorectal cancer, whereas no patient died of another cause. Figure 7.1 shows that the colorectal cancer-specific survival did not significantly differ between the studied groups; the colorectal cancer-specific survival at 4 years was 83% in the control group and 72% in the donation group (p=0.30). Of the 121 patients, who underwent curative surgery, 15 of the 85 patients in the control group and 6 of the 36 patients in the donation group had recurrent disease during follow-up.

Figure 7.1. Colorectal cancer-specific survival in all 135 patients after colorectal cancer surgery without transfusions.

The survival at 4 years was 83% in the control group and 72% in the donation group (p=0.30).

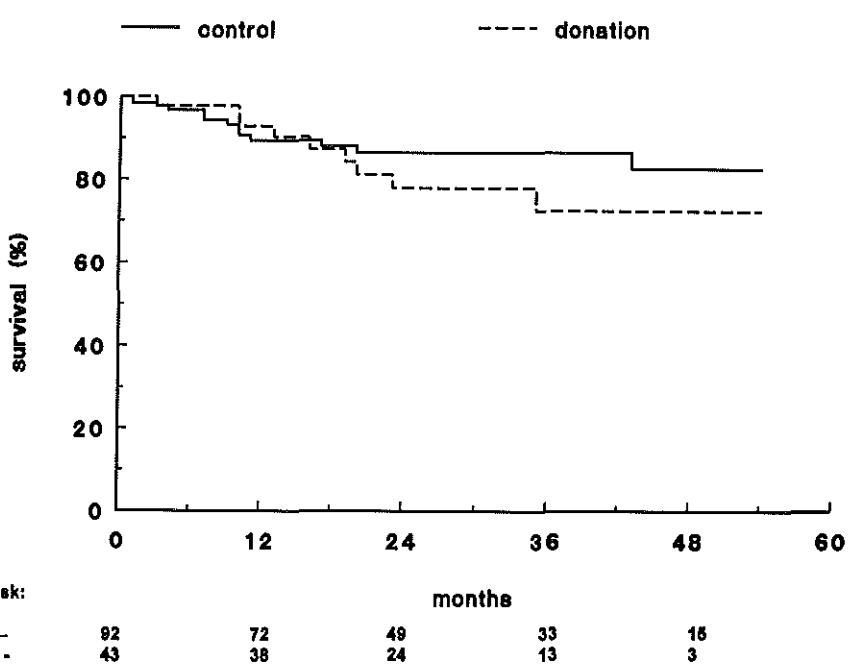


Table 7.3. Multivariate analysis of various factors with respect to recurrence rate of the patients who underwent curative surgery without transfusions (N=121).

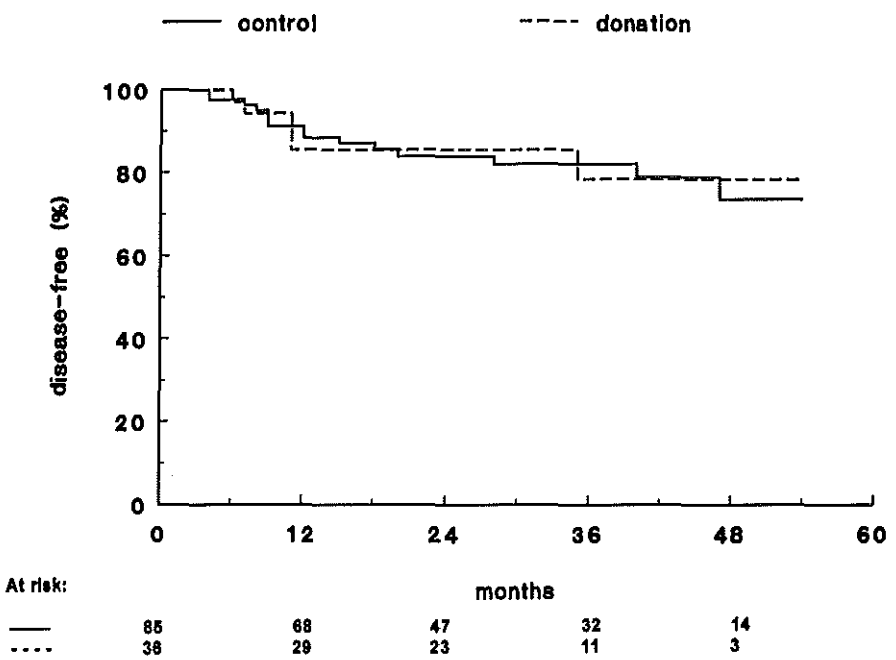
Factor	Relative recurrence rate	95% confidence interval	p value
Study group			
Control group	1		
Donation group	0.9	0.3 - 2.6	0.81
Age*	1.0	0.9 - 1.0	0.18
Sex			
Male	1		
Female	0.7	0.3 - 1.9	0.52
Dukes' classification			
A	1		
B	3.0	0.3 - 26.8	0.33
C	14.5	1.8 - 118.6	0.01
Tumor size*	1.1	0.9 - 1.3	0.32
Differentiation			
Well	1		
Moderate	1.1	0.2 - 5.9	0.94
Poor	1.9	0.2 - 14.7	0.56
Operation			
Intra-abdominal	1		
Rectal involvement	1.1	0.4 - 3.1	0.78
Adjacent organ fixation			
No	1		
Yes	0.3	0.03 - 2.4	0.25

* As compared to 1 year younger (age), or 1 cm smaller (tumor size).

Figure 7.2 indicates that the disease-free survival of the 121 patients, who underwent curative surgery, were not significantly different between both groups; the disease-free survival at 4 years was 74% in the control group and 78% in the donation group (p=0.94). Table 7.3 shows the results of Cox regression of various factors with respect to the recurrence rate of the patients who underwent curative surgery (N=121). Study group was not a factor affecting the recurrence rate (p=0.81). The only significant factor influencing colorectal cancer prognosis in these patients was the Dukes' classification.

Figure 7.2. Disease-free survival of 121 patients who underwent curative surgery for colorectal cancer without transfusions.

The disease-free survival at 4 years was 74% in the control group and 78% in the donation group (p=0.94).



Discussion

This study shows that donation of two units of blood preoperatively, did not alter the recurrence rate and the survival of colorectal cancer patients. So far, no data are available of the effects of blood donation in cancer patients. The only published reports investigated the incidence of cancer among healthy blood donors. Merk et al.[10] found in a study of 37,795 Swedish blood donors that the incidence of cancer was significantly less as compared to the expected value. The relative risk was 0.79 for donors (p<0.001). As an

explanation for these findings, which were in sharp contrast with their primarily hypothesis, the authors postulated that the results might be influenced by selection of the blood donors. Lasek et al.[11] found a long lasting depression of the natural killer cell activity after long-term blood donations, and recently came to the conclusion that these immunological changes did not bear any clinical consequence with respect to the incidence of cancer. In a study among 3126 voluntary blood donors in Poland no significant difference in the observed and expected number of cancer cases (all types) was found. They observed an increased risk of developing liver cancer, but it seems unlikely that this is a reflection of depressed natural killer cell activity among those patients. Tartter et al.[12] have found that a low natural killer cell activity is related to an increased risk of colorectal cancer recurrence. Although the exact role of natural killer cells is unclear, this might be a result of their immune-surveillance against circulating cancer cells.[13,14] Because blood donation decreases natural killer cell activity significantly, it was questionable whether this procedure is as safe in colorectal cancer patients as it is in healthy blood donors. The results of this study clearly shows that the impaired function of natural killer cells induced by blood donation had no clinical impact on the prognosis of colorectal cancer patients.

With respect to the risk of infectious complications after colorectal cancer surgery, we did also not observe any effect of blood donation. In a earlier study we found that the risk of infections was affected by the location of the tumor and an increased age.[15] Autologous blood transfusions did not decrease the rate of infectious complications as compared to allogeneic transfusions. Heiss et al.[16] suggested in a similar but smaller study that patients in the autologous group had less infections. Whether this marginally significant difference was a result of the different effects of allogeneic and autologous blood transfusions was unclear. Another explanation stated by the authors was that the donation of blood could have a protective effect against bacterial infections in the postoperative period. We performed multivariate analysis allowing for various potential risk factors, including also whether blood was donated or not. Using this method there was no significant difference between these two groups, which made us conclude that preoperative donation of blood in colorectal cancer surgery does not affect the risk of infectious complications.

Jensen et al.[17] found that an impaired natural killer cell activity is related to an increased rate of wound infections after colorectal surgery. They have found a decreased natural killer cell activity induced by transfusions of whole blood as compared to leukocyte-depleted blood. Although the overall rate of infections was similar in both groups, the patients transfused with whole blood had more wound infections as compared to the patients transfused with filtered blood. In a larger study by Houbiers et al.[18] comparing leukocyte-depleted blood with buffy-coat depleted blood no differences in infectious complications were found. We did not find that a decreased natural killer cell function, due to blood donation, had any impact on the rate of infections, but this might be

otherwise when the immunological changes are induced by transfusions. The effects of transfusions or donation on natural killer cell function and other immunological parameters might be totally different. In the present study we have not analyzed the effects of transfusions on natural killer cell function because all transfused patients were excluded, but the impairment of natural killer cell function induced by blood donation did not affect the risk of infectious complications after colorectal cancer surgery.

References

1. Blumberg N, Heal JM. Effects of transfusion on immune function; cancer recurrence and infection. Arch Pathol Lab Med 1994; 118:371-9.
2. Lasek W, Plodziszewska M, Jakobisiak M. The effect of blood donation on natural killer activity in man. J Clin Lab Immunol 1987; 22:165-8.
3. Marquet RL, Hoynck van Papendrecht MA, Busch ORC, Jeekel J. Blood donation leads to a decrease in natural killer cell activity: a study in normal blood donors and cancer patients. Transfusion 1993; 33:368-73.
4. Busch ORC, Hop WCJ, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 328:1372-6.
5. Council on Scientific Affairs. Autologous blood transfusions. JAMA 1986; 256:2378-80.
6. Turnbull RB, Kyle K, Watson FR, Spratt J. Cancer of the colon: The influence of the no-touch isolation technic on survival rates. Ann Surg 1967; 166:420-5.
7. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457-81.
8. Cox DR. Regression models and life tables. J R Stat Soc [B] 1972; 34:187-202.
9. Cox DR. The analysis of binary data. 1970 Methuen London.
10. Merk K, Mattson B, Mattson A, Holm G, Gullbring B, Björkholm M. The incidence of cancer among blood donors. Int J Epidemiol 1990; 19:505-9.
11. Lasek W, Jakobisiak M, Stoklosa T. Decreased natural killer cell activity in whole-blood donors does not seem to result in increased cancer incidence. Transfusion 1994; 34:359-60.
12. Tartter PI, Steinberg B, Barron DM, Martinelli G. The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. Arch Surg 1987; 122:1264-8.
13. Ritz J. The role of natural killer cells in immune surveillance. N Engl J Med 1989; 320:1748-9.
14. Oldham RK. Natural killer cells: history, relevance and clinical application. Nat Immun Cell Growth Regul 1990; 9:297-312.
15. Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Autologous blood and infections after colorectal surgery. Lancet 1994; 343:668-9.
16. Heiss MM, Mempel W, Jauch KW, et al. Beneficial effect of autologous blood transfusion on infectious complications after colorectal cancer surgery. Lancet 1993; 342:1328-33.
17. Jensen LS, Andersen AJ, Christiansen PM, et al. Postoperative infections and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. Br J Surg 1992; 79:513-6.
18. Houbiers JGA, Brand A, van de Watering LMG, et al. Randomised controlled trial comparing transfusion of leucocyte-depleted or buffy-coat-depleted blood in surgery for colorectal cancer. Lancet 1994; 344:573-78.

CHAPTER 8

GENERAL DISCUSSION

Introduction

Blood transfusions have an immunosuppressive effect, which is beneficial for patients receiving a kidney transplantation. It is suggested that this transfusion-induced immunosuppression may have detrimental effects in patients operated for cancer.[1] Due to allogeneic blood transfusions in the perioperative period the prognosis of cancer patients might be impaired, and the rate of postoperative infectious complications might be increased. Several retrospective studies indeed found that these deleterious effects occur in colorectal cancer patients. Experimental studies showed that growth of certain tumors was enhanced after allogeneic blood transfusions, which suggests that the relationship between blood transfusions and impaired cancer survival might be causal. However, there are also conflicting data from both experimental and clinical studies. Transfusions are given either because of the disease or as a result of surgical treatment. Therefore it is questionable, whenever a relationship between transfusions and cancer prognosis would exist, whether this relationship is causal or indirect. The need for transfusion could be an indicator of other prognostic factors, which are unknown, and therefore blood transfusion is coincidentally related to prognosis. The only way to resolve the question of causality is to perform a randomized trial comparing transfused patients with patients who did receive transfusions. Unfortunately, it is disputable whether such a trial design is ethically justified. Although the causality of the putative relationship can not be resolved completely it is possible to investigate whether the effects of allogeneic blood transfusions can be overcome. Therefore, we performed this randomized multicenter clinical trial in colorectal cancer patients to determine whether predeposit autologous blood transfusions would improve the prognosis and decrease the rate of infectious complications as compared with standard allogeneic blood transfusions (without buffy coat).

Postoperative infectious complications

Immunosuppression is linked to an increased susceptibility to infections and therefore it is conceivable that transfusion-induced immunosuppression is correlated with an increased rate of postoperative infectious complications. However, as discussed in more extent in chapter 5 we could not find support for a causal relationship between blood transfusions of any type (allogeneic or autologous) with infections. We only found an increased rate of infections when more than two units of blood were required, which is to our opinion supportive for the conclusion that an extensive surgical trauma and related blood loss is the real factor which affects the risk of infectious complications after colorectal cancer surgery. We will focus the general discussion on the putative detrimental relationship between blood transfusions and colorectal cancer prognosis.

Colorectal cancer prognosis

This study shows that, although a remarkable reduction in the exposure to allogeneic blood transfusions was achieved by a predeposit autologous blood donation program, this reduction did neither decrease the rate of colorectal cancer recurrence or improved the survival of those patients. These findings led to the conclusion that there is no oncologic reason to use a predeposit autologous blood donation program in colorectal cancer patients. The study by Heiss et al.[2] which had a comparable study design had too less patients (N=120) to draw reliable conclusions. The preliminary results of their intention-to-treat analysis revealed also no benefits for patients randomized to the autologous group with regard to cancer prognosis (survival; p=0.204, recurrence rate; p=0.11). A not randomized study found no differences in survival comparing autologous and allogeneic blood transfusions in patients who underwent radical surgery for prostate cancer.[3] Another randomized study in colorectal cancer had a different study design to overcome the putative detrimental effects of allogeneic transfusions and was recently published by Houbiers et al.[4] Based on the findings that the transfusion-induced immunosuppression might be mediated by the transfused leukocytes,[5] it was postulated that these effects might be prevented by removing the leukocytes as much as possible. Houbiers found in colorectal cancer patients no advantage on prognosis in the patients who had received leukocyte depleted blood as compared with those patients who had received allogeneic transfusions (without buffy coat). The authors concluded that, although there is no reason to transfuse leukocytes, there is no oncologic reason to make an effort to remove more than the buffy coat alone.

To explore the relationship between blood transfusions and colorectal cancer prognosis our study was regarded as a nonrandomized study. In agreement with several retrospective studies it was demonstrated that the transfused patients had a significantly worse prognosis as compared with the patients who did not receive transfusions. The disease-free survival at 4 years was 59% and 73%, respectively. In multivariate analysis the relationship between transfusions and prognosis was about the same when the different types of transfusions (allogeneic and autologous) were compared. Because the transfusion of autologous blood was limited to two units subgroup analyses were performed by excluding the patients who received more than two transfusions. This multivariate analysis also showed an equal effect of allogeneic and of autologous transfused patients as compared with the patients who did not require transfusions. These findings may suggest that there is indeed a direct effect of allogeneic blood transfusions on prognosis, and that this effect also exists for autologous transfusions.

Immunomodulation by autologous blood transfusion

Autologous blood transfusions in humans are comparable to syngeneic transfusions in animals. In the animal experiments showing a deleterious effect of allogeneic transfusions

on tumor growth no such effect was found when syngeneic blood transfusions were given.[6,7,8] Although there was no experimental support for an effects of autologous transfusions on tumor growth, it was found that autologous transfusions could have an immunomodulatory effect as well. Recently, Perttilä et al.[9] found that the immunological changes after open heart surgery did not differ significantly between autologous, obtained by a cell saver, or allogeneic blood transfusions. These findings were however contradicted by Peller et al.[10] who found that the transfusion of autologous blood improved impaired immune responses in cancer patients, whereas allogeneic transfusions did not. As a result of storage blood contains microaggregates of fibrin strands and degenerating leukocytes and platelets which bind to fibronectin. Fibronectin has a role in macrophage function. Due to the transfusion of stored blood (allogeneic or autologous) the fibronectin concentration is reduced in the recipient and therefore related immune functions may be impaired. Lawrance et al.[11] found that after storage of blood platelet derived growth factors were increased, which affected tumor growth. The storage of blood has also great impact on the synthesis of prostaglandin E_2 which may impair immune responses in the recipient.[12] Furthermore, the mitogenic activity of plasma is increased with a longer storage time.[13] This increment of mitogenic activity becomes apparent after a storage time of about two weeks.

Immunomodulation by transfusion of plasma

The suggestion that the negative effects of blood transfusions were due to factors present in plasma was supported by clinical studies which found that the transfusion-induced effects were a result of the plasma transfusion.[14,15] In the study presented in this thesis the number of patients who received fresh-frozen plasma was too small to justify firm conclusions. A marked effect of packed cells (buffy coat depleted) was demonstrated. The recurrence rate for patients receiving allogeneic transfusions was 2.3, and for patients receiving autologous transfusions was 1.8 as compared with patients who did not require transfusions. In a recent meta-analysis a relationship between blood transfusions and poor prognosis of the same magnitude as our results was reported.[16] The cumulative odds-ratio of negative outcomes after perioperative blood transfusions in this meta-analysis was 1.80 for recurrent disease. However, no effects of fresh-frozen plasma were found in this study.

Blood transfusion and local recurrence

The negative relationship between blood transfusions and colorectal cancer recurrence was unraveled in more extent by subdividing the endpoint of cancer recurrence into local recurrence and distant metastases. It was found that there was no relationship between blood transfusions of any type (allogeneic, autologous, or both types) and the incidence of distant metastases. However, the risk of having local recurrence was significantly increased for patients who received blood transfusions of any kind. The risk of local recurrence did not differ significantly between the different types of transfusions.

Obviously, local failure in colorectal cancer is more common in rectal cancer than in colonic cancer and rectal surgery is also associated with a greater need for transfusions. When the factor 'tumor location in the rectum' was investigated in this study, indeed this factor affected the risk of local recurrence. But, when multivariate analysis allowing also for tumor location was performed, no additional effect on prognosis of this factor was found. The need for blood transfusions was an independent factor affecting the risk of local recurrence.

Local recurrence is related to surgical failure which suggests that the relationship between blood transfusions and impaired prognosis is not causal and is not induced by immunosuppression, but that this is an indirect association. Therefore, we conclude that not the blood transfusions themselves but rather the circumstances necessitating transfusion are the real determinants of prognosis in colorectal cancer patients.

Blood loss and cancer recurrence

Speculations can be made which circumstances determine the need for transfusions. The most important factor is probably the amount of blood loss during surgery. Blood loss itself can induce immunosuppression.[17,18] And, it is also found that blood loss can stimulate tumor growth in animal experiments.[19] To elucidate the effect of blood loss separately from the effect of blood transfusions in our randomized study is difficult, because, as a result of the transfusion rules, blood transfusions are strongly correlated to blood loss. In experimental studies blood loss without surgical trauma can be imitated by blood donation. Since in our randomized study we used predeposit autologous blood as an alternative for allogeneic transfusions, the patients randomized to the autologous group had to donate blood. The investigations on the putative immunological changes provoked by blood donation (blood loss without surgical trauma) revealed that the NK cell activity was significantly decreased after blood donation in colorectal cancer patients. When taken into account only those patients who did not require any transfusion (packed cells or plasma, autologous or allogeneic), the clinical consequences of this impaired NK cell function could be investigated. No significant impact of blood donation on the prognosis of colorectal cancer patients could be demonstrated in this study.

As discussed above it is unlikely that immunological changes only affect the risk of local recurrence and not the risk of distant metastases. More likely is the explanation that blood loss and the need for blood transfusions are surrogate markers of surgical extent or trauma. This possible explanation of the negative association between transfusions and prognosis was already given by Hodgson and Lowenfels in 1982.[20]

Whether this view on the topic can explain all the findings from previous experimental and clinical studies will be further discussed.

Concluding remarks

The finding that some animal models showed a tumor promoting effect of blood transfusions suggests that there indeed might be a clinical situation in which there is a direct effect of blood transfusions on tumor recurrence. This clinical situation could be present in only a specific tumor-host relationship and therefore difficult to find in clinical studies. The same variety in results was already found in animal experiments investigating the effect of blood transfusions and transplant survival. Although in clinical organ transplantation overall survival is increased by blood transfusions, stratification into subgroups revealed that about 30% of the patients respond with sensitization. Results from specific animal models already had indicated that this could have been anticipated. Thus, the variety in results from (inbred) animals reflects the variety in results that may occur in an outbred population. Therefore, the different findings from animal experiments provide information about the biological variation of a certain phenomenon. The whole spectrum of this variability may occur in a clinical situation, however, it is difficult to pinpoint where and when.

The fact that so many controversial clinical reports were published suggests that whenever an association between blood transfusions and colorectal cancer prognosis exists it probably will be indirect and not causal. One of the reasons why those studies gave different outcomes might be that they also reflect the variability of the phenomena that may occur. It is also possible that the presence of a transfusion effect depends on the indication for transfusion, which can vary enormously between different centers. The controversy in clinical findings might also be explained by the variability among surgeons or tumors.[21] These tumor and surgeon related differences might influence the need for blood transfusions as well as the incidence of recurrent disease. Therefore, a negative association is found in some studies whereas this is not the case in others, which makes it more likely that the relationship between blood transfusions and colorectal cancer prognosis is noncausal but simply indirect. Furthermore, the suggestion that transfusion-induced immunosuppression could influence the prognosis of colorectal cancer patients is based on the believe that tumor growth might be affected by subtle immunological changes. However, human colorectal cancer is not an immunogenic tumor and has so far not shown to be affected by immunosuppression. Although, some tumors occur more frequently in patients who received long-lasting immunosuppression this did not concern colorectal carcinoma.[22,23] Additionally, immunotherapy reflecting sensitivity to immune responses in colorectal cancer had only marginal effects on prognosis.[24,25]

Putting together all the information coming from experimental studies, retrospective clinical studies, and randomized clinical studies which investigated whether blood transfusions adversely affect colorectal cancer prognosis we conclude that whenever such a relationship exists it is noncausal. The sobering final conclusion therefore is that not the blood transfusions themselves but rather the circumstances necessitating transfusion are the real determinants of prognosis in colorectal cancer.

References

1. Gantt CL. Red blood cells for cancer patients. Lancet 1981; 2:363.
2. Heiss MM, Mempel W, Delanoff C, et al. Blood transfusion-modulated tumor recurrence: first results of a randomized study of autologous versus allogeneic blood transfusion in colorectal cancer patients. J Clin Oncol 1994; 12:1859-67.
3. Ness PM, Walsh PC, Zahurak M, Baldwin ML, Piantadosi S. Prostate cancer recurrence in radical surgery patients receiving autologous or homologous blood. Transfusion 1992; 32:31-6.
4. Houbiers JGA, Brand A, van de Watering LMG, et al. Randomised controlled trial comparing transfusion of either leucocyte-depleted or buffy-coat-depleted blood in colorectal cancer surgery. Lancet 1994; 344:573-8.
5. Persijn GG, Cohen B, Lansbergen Q, van Rood JJ. Retrospective and prospective studies on the effect of blood transfusions in renal transplantation in the Netherlands. Transplantation 1979; 28:396-401.
6. Francis DMA, Shenton BK. Blood transfusion and tumour growth: evidence from laboratory animals. Lancet 1981; 2:871.
7. Marquet RL, de Bruin RWF, Dallinga R, Singh SK, Jeekel J. Modulation of tumor growth by allogeneic blood transfusion. J Cancer Res Clin Oncol 1986; 111:50-3.
8. Singh SK, Marquet RL, de Bruin RWF, Westbroek DL, Jeekel J. Promotion of tumor growth by blood transfusions. Transplant Proc 1987; 19:1473-4.
9. Perttilä JT, Salo MS, Jalonen JR, Kuttila KT, Viinamäki O, Pulkki KJ. Blood transfusions with autologous and leukocyte-depleted or standard allogeneic red blood cells and the immune response to open heart surgery. Anesth Analg 1994; 79:654-60.
10. Peller S, Sayfan J, Levy Y, et al. Immunological profile changes following perioperative autologous vs. homologous blood transfusion in oncologic patients. J Surg Oncol 1994; 56:98-101.
11. Lawrance RJ, Cooper AJ, Loizidou M, Alexander P, Taylor I. Blood transfusion and recurrence of colorectal cancer: the role of platelet derived growth factors. Br J Surg 1990; 77:1106-9.
12. Ross WB, Leaver HA, Yap PL, Raab GM, Su BH, Carter DC. Prostaglandin E_2 production by rat peritoneal macrophages: role of cellular and humoral factors in vivo in transfusion-associated immunosuppression. FEMS Microbiol Immunol 1990; 2:321-5.
13. Hoh H, Umpleby H, Cooper A, Taylor I. Recurrence of colorectal cancer and perioperative blood transfusion; is blood storage time important? Dis Colon Rectum 1990; 33:127-30.
14. Blumberg N, Heal JM, Murphy P, Agarwal MM, Chuang C. Association between transfusion of whole blood and recurrence of cancer. Br Med J 1986; 293:530-3.
15. Marsh J, Donnan PT, Hamer-Hodges DW. Association between transfusion with plasma and recurrence of colorectal carcinoma. Br J Surg 1990; 77:623-6.
16. Chung M, Steinmetz OK, Gordon PH. Perioperative blood transfusion and outcome after resection for colorectal carcinoma. Br J Surg 1993; 80:427-32.
17. Chaudry IH, Ayala A. Mechanism of increased susceptibility to infection following hemorrhage. Am J Surg 1993; 165:59-67.
18. Hoynck van Papendrecht MAW, Busch ORC, Jeekel J, Marquet RL. The influence of blood loss on tumour growth: effect and mechanism in an experimental model. Neth J Surg 1991; 43:85-8.
19. Singh SK, Marquet RL de Bruin RW, Hop WCJ, Westbroek DL, Jeekel J. Consequences of blood loss on growth of artificial metastases. Br J Surg 1988; 75:377-9.
20. Hodgson WJB, Lowenfels AB. Blood transfusions and recurrence rates in colonic malignancy. Lancet 1982; 2:1047.
21. McArdle CS, Hole D. Impact of variability among surgeons on postoperative morbidity and mortality and ultimate survival. Br Med J 1991; 302:1501-5.
22. Penn I. Cancers after cyclosporine therapy. Transplant Proc 1988; 20:276-9.
23. Penn I. The changing pattern of posttransplant malignancies. Transplant Proc 1991; 23:1101-3.
24. Rosenberg SA, Yang JC, Topalina SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high dose bolus interleukin-2. JAMA 1994; 271:907-13.
25. Hoover HC, Brandhorst JS, Peters LC, et al. Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase-III prospectively randomized trial. J Clin Oncol 1993; 11:390-9.

Summary and conclusions

Chapter 1 is the general introduction of this thesis and gives and overview of the literature related to the risks of blood transfusions. Especially, the induction of immunosuppression is discussed in relation to transplantation, cancer prognosis, and infectious complications after surgery. The relationship between blood transfusions and immunosuppression is, obviously, present and beneficial in patients receiving a transplantation. However, the question whether this transfusion-induced immunosuppression has a negative effect on prognosis or on infectious complications after colorectal surgery is not clear from experimental or clinical (mostly retrospective) studies so far. Therefore, there is a need for randomized studies to investigate whether this putative detrimental effect of allogeneic blood transfusions could be overcome. We have chosen to use a predeposit autologous blood donation program as an alternative for allogeneic blood transfusion. At the end of chapter 1 the aims of the study are presented.

Chapter 2 gives in extent the material and methods of the randomized multicenter trial in which the value of a predeposit autologous blood transfusion program in colorectal cancer patients is investigated. The results of the study are given regarding the intention-to-treat principle. The colorectal cancer-specific survival at 4 years was 67% in the allogeneic group and 62% in the autologous group (p=0.39). Among the 423 patients who underwent curative surgery, 66% of those in the allogeneic group and 63% of those in the autologous group had no recurrence of colorectal cancer at 4 years (p=0.93). It was concluded that a significant reduction of exposure to allogeneic blood transfusions was achieved by our donation program. However, no significant differences with regard to survival (overall or colorectal cancer specific) or disease-free survival were found for patients randomized to either the allogeneic or autologous group. Although the transfused patients had a worse prognosis as compared with patients who did not receive transfusions, there was no difference between the patients receiving either 1 or 2 allogeneic transfusions or 1 or 2 autologous transfusions. The relative risks of recurrence were 2.1 and 1.8, respectively.

Chapter 2: 'Letters to the editor' presents two letters which were published as a reaction to chapter 2. An authors reply was given in which the effects of fresh-frozen plasma on colorectal cancer prognosis is given. No detrimental effects of either allogeneic or autologous plasma transfusions on prognosis were demonstrated in this study. It is, however, important to realize that the number of patients receiving fresh-frozen plasma was too small to allow reliable conclusions.

Chapter 3 elaborates more on the worse prognosis for transfused patients as compared with patients who did not receive transfusions. The disease-free survival at 4 years were 59% and 73%, respectively. Multivariate analysis, allowing for various prognostic factors, showed that there was no difference in prognosis between the randomized groups. The only factors influencing the rate of recurrence were the Dukes' classification and whether blood transfusions were given or not. This analysis also revealed that patients receiving only allogeneic or only autologous transfusions, and patients receiving both types of transfusions all had a significant increased risk of recurrence as compared with patients who did not require transfusions. No significant differences in prognosis were found in comparing these three groups of transfused patients.

Chapter 4 unravels in more extent the negative association found in the previous chapter. When recurrent disease was subdivided into local recurrence and distant metastases it was found that there was no relationship between blood transfusions and the incidence of patients having distant metastases. However, there was a significant higher incidence of patients having local recurrence in the transfused groups of patients. Multivariate analysis, allowing also for Dukes' classification and tumor location showed that the relative risk of local recurrence was 5.2 in the transfused group of patients. A significantly increased risk of local recurrence was found for all the transfused patients, irrespective of the type (allogeneic, autologous, or both types) of blood transfusions given. Because local recurrences seems to be related to surgical failure, it was concluded that not the blood transfusions themselves but rather the circumstances necessitating them are the real predictors of prognosis in colorectal cancer.

Chapter 5 shows the results of the randomized study with regard to the rate of postoperative infectious complications. It was found that the incidence of postoperative infections after colorectal cancer surgery was not significantly different between allogeneic and autologous group. In the allogeneic group 25% of the patients had infections as compared with 27% in the autologous group. Because the incidence of infections can vary per hospital the influence of the multicentricity of the study was investigated. We did not find any effect of this with regard to infections.
It also was found that patients who received more than two units of blood had an significantly increased risk of infections as compared with patients receiving 1 or 2 transfusions or no transfusion at all. When two units, irrespective whether they were of the allogeneic or autologous type, were transfused there was no increase in the risk of infections as compared with the patients who did not require transfusions. We conclude that the risk of infections is not causally related to transfusions but that the circumstances necessitating transfusions increase the risk of infections.

Chapter 6 presents immunological studies to explore the effect of blood donation in healthy blood donors and in colorectal cancer patients. No effect of donation was found on the proportion of B, T, and NK cells assessed by flow cytometric analysis. T cell function was investigated by PHA stimulation and appeared not to be affected by blood donation. However, a significant depression of NK cell activity after donation was found in normal blood donors and in colorectal cancer patients. Colorectal cancer patients had after donation of two units of blood 54% of the NK cell activity left as before donation. Therefore, the NK cell activity of the patients in the autologous group was significantly less as compared with the patients in the allogeneic group.

Chapter 7 deals with the clinical implication of the immunological changes in colorectal cancer patients who donated two units of blood for autologous transfusions. To evaluate the effect of donation, all the patients who needed transfusion were excluded from this analysis. It was found that the risk of colorectal cancer recurrence and the survival was not affected by blood donation. The disease-free survival at 4 years was 78% in the donation group, whereas it was 74% in the group of patients who did not donate blood.
It also was shown that patients who donated blood had no increased risk of infectious complications after surgery as compared with the patients who did not donate blood. The rate of infections was 16% in the donation group as compared with 22% in the group of patients who did not donate blood. Therefore we conclude that, although the NK cell function was lower in patients who donated blood, this had no clinical implication with regard to prognosis and infection.

Chapter 8 is the general discussion of this thesis. It gives and overview of the results of the studies presented in this thesis. The results and the putative explanations for the findings are discussed.

Conclusions

1. A predeposit autologous blood transfusion program did neither improve the survival nor reduced the recurrence rate in colorectal cancer patients.
2. Although a reduction of exposure to allogeneic blood is of benefit, there is no oncologic reason to use a predeposit autologous blood donation program in colorectal cancer patients.
3. The association between blood transfusions and colorectal cancer prognosis is the same for allogeneic as for autologous blood.

4. The risk of local recurrence is associated with the need for blood transfusions, whereas the risk of distant metastases is not.
5. Not the blood transfusions themselves but rather the circumstances necessitating transfusion are the real determinants of prognosis in colorectal cancer patients.
6. A predeposit autologous blood transfusion program does not reduce the rate of infectious complications after colorectal cancer surgery.
7. The need for more than two units of blood increased the risk of infectious complications, not mattering whether the first two transfusions were allogeneic or autologous.
8. Although blood donation leads to a decreased natural killer cell activity in colorectal cancer patients, this impaired natural killer cell function had not clinical impact on prognosis and on the rate of postoperative infectious complications.

Samenvatting en conclusies

Hoofdstuk 1 is de algemene introductie van dit proefschrift en geeft een overzicht van de literatuur over de risico's van bloedtransfusies. Besproken wordt met name het induceren van immunosuppressie in relatie tot transplantatie, oncologische prognose en postoperatieve infectieuze complicaties. De relatie tussen bloedtransfusies en immunosuppressie is heel duidelijk aanwezig en gunstig voor patiënten die een transplantatie ondergaan. De vraag echter of deze immunosuppressie een negatief effect heeft op de prognose en de infectieuze complicaties na colorectale chirurgie is door experimentele en klinische (meestal retrospectieve) studies tot nu toe niet duidelijk geworden. Daarom was er behoefte aan gerandomiseerd onderzoek om na te gaan of deze mogelijk negatieve effecten van allogene bloedtransfusies voorkomen kunnen worden. Wij hebben gekozen om preoperatief gedoneerde autologe bloedtransfusies te gebruiken als alternatief voor allogene transfusies. Aan het eind van hoofdstuk 1 worden de doelstellingen van de studie uiteengezet.

Hoofdstuk 2 bespreekt uitgebreid de opzet van de gerandomiseerde multicenter studie waarin de waarde van een autoloog bloedtransfusie programma in patiënten met colorectaal carcinoom wordt onderzocht. De resultaten van de studie worden gegeven met betrekking tot het intention-to-treat principe. De overleving na 4 jaar was 67% in de allogene groep en 62% in de autologe groep (p=0.39). Van de 423 patiënten die curatief geopereerd werden had 66% in de allogene groep en 63% in de autologe groep na 4 jaar geen recidief van het colorectale carcinoom (p=0.93). Er wordt geconcludeerd dat een significante reductie van blootstelling aan allogene bloedtransfusies was bereikt met ons donatie programma. Er werden echter geen significante verschillen in overleving of in ziektevrije overleving gevonden tussen de patiënten die gerandomiseerd waren voor de allogene of voor de autologe groep. Hoewel de getransfundeerde patiënten een slechtere prognose hadden dan de patiënten die niet getransfundeerd werden, waren er geen verschillen tussen de patiënten die 1 of 2 allogene of die 1 of 2 autologe transfusies kregen. Het relatieve risico op een recidief was respectievelijk 2,1 en 1,8.

Hoofdstuk 2: 'Letters to the editor' bevat 2 brieven die werden gepubliceerd als een reactie op hoofdstuk 2. Er wordt een 'authors reply' gegeven waarin de effecten van fresh-frozen plasma op de prognose van patiënten met een colorectaal carcinoom worden besproken. In deze studie worden geen negatieve effecten op de prognose gevonden van zowel allogeen als van autoloog plasma. Het is echter wel belangrijk om zich te realiseren dat de aantallen patiënten die plasma kregen te klein was om betrouwbare conclusies te trekken.

Hoofdstuk 3 behandelt uitvoeriger de slechtere prognose van getransfundeerde patiënten vergeleken met patiënten die niet getransfundeerd werden. De ziektevrije overleving na 4 jaar was respectievelijk 59% en 73%. Multivariate analyse, rekening houdend met verschillende prognostische factoren, liet geen verschil in prognose zien tussen de gerandomiseerde groepen. De Dukes' classificatie en het al dan niet krijgen van bloedtransfusies waren de enige factoren die de recidiefkans beïnvloedden. Deze analyse toonde ook dat patiënten die alleen allogene of alleen autologe transfusies kregen en patiënten die beide soorten transfusies kregen allen een significant hogere kans op een recidief hadden dan de patiënten die geen transfusies nodig hadden. Er werd geen significant verschil gevonden tussen deze drie groepen getransfundeerde patiënten.

Hoofdstuk 4 onderzoekt uitvoeriger de negatieve relatie welke in het vorige hoofdstuk werd gevonden. Als recidief wordt onderverdeeld in lokaal recidief en afstandsmetastasen, dan wordt er geen relatie gevonden tussen bloedtransfusies en de kans op afstandsmetastasen. Er wordt echter wel een significant hogere kans op het krijgen van een lokaal recidief gevonden in de groep patiënten die getransfundeerd werden. Multivariate analyse, rekening houdend met de Dukes' classificatie en tumor lokalisatie, toonde dat het relatieve risico op lokaal recidief 5,2 was in de groep patiënten die getransfundeerd werden. Een significant verhoogd risico op lokaal recidief werd gevonden in alle groepen patiënten die transfusies kregen. Dit verhoogde risico was onafhankelijk van het soort bloedtransfusie (allogeen, autoloog of beide soorten). Omdat de kans op een lokaal recidief gerelateerd lijkt aan chirurgisch falen, wordt geconcludeerd dat niet de bloedtransfusies zelf maar de omstandigheden die transfusies nodig maken de prognose bij het colorectaal carcinoom bepalen.

Hoofdstuk 5 toont de resultaten van de gerandomiseerde studie met betrekking tot de incidentie van postoperatieve infectieuze complicaties. Er werd gevonden dat de incidentie van postoperatieve infectieuze complicaties na colorectale chirurgie niet significant verschilde tussen de allogene en autologe groep. In de allogene groep had 25% van de patiënten infecties vergeleken met 27% in de autologe groep. Omdat de kans op infecties per ziekenhuis kan verschillen werd de invloed van multicentriciteit van de studie onderzocht. Wij konden geen invloed hiervan op de infectiekans vinden.
Er werd ook gevonden dat de patiënten die meer dan 2 eenheden bloed kregen een significant hogere kans op infecties hadden vergeleken met patiënten die 1 of 2 transfusies of helemaal geen transfusies kregen. Wanneer 2 eenheden getransfundeerd werden, onafhankelijk of deze allogeen of autoloog waren, was er geen verhoogde infectiekans vergeleken met de patiënten die geen transfusies kregen. Wij concluderen dat de kans op infectieuze complicaties niet causaal gerelateerd is aan bloedtransfusies, maar dat de omstandigheden die transfusies nodig maken de kans op infecties vergroot.

Hoofdstuk 6 geeft de immunologische studies die het effect van bloeddonatie onderzoeken in gezonde bloeddonoren en in patiënten met een colorectaal carcinoom. Donatie heeft geen effect op de verhoudingen van B, T, en NK cellen, welke bepaald werden met flow cytometrie. De T cel functie werd onderzocht middels PHA stimulatie en bleek niet beïnvloed te worden door bloeddonatie. Er werd echter een significante daling van de NK cel activiteit na donatie gevonden in normale bloeddonoren en in patiënten met een colorectaal carcinoom. Patiënten met een colorectaal carcinoom hadden na donatie van 2 eenheden bloed nog 54% van de NK cel activiteit van voor donatie over.

Hoofdstuk 7 behandelt de klinische implicaties van de immunologische veranderingen in patiënten met een colorectaal carcinoom die 2 eenheden bloed hebben gedoneerd voor autologe transfusie. Om het effect van bloeddonatie te evalueren werden alle patiënten die transfusies nodig hadden uitgesloten van deze analyse. Er werd gevonden dat het risico op recidief en de overleving niet werden beïnvloed door bloeddonatie. De ziektevrije overleving na 4 jaar was 78% in de groep patiënten die doneerden, terwijl dit 74% was in de groep patiënten die niet doneerden.
Er werd ook gevonden dat patiënten die bloed doneerden geen hogere kans hadden op het krijgen van postoperatieve infectieuze complicaties dan patiënten die niet doneerden. Het percentage infectie was 16% in de donatie groep vergeleken met 22% in de groep patiënten die geen bloed gaf. Daarom concluderen wij dat, hoewel de NK cel activiteit lager is in patiënten die bloed doneerden, dit geen klinische gevolgen heeft met betrekking tot de prognose en de infectiekans.

Hoofdstuk 8 is de algemene discussie van dit proefschrift. Het geeft een overzicht van de resultaten van de studies uit dit proefschrift. De resultaten en de mogelijke verklaringen voor de bevindingen worden uiteengezet.

Conclusies

1. Een preoperatief autoloog bloedtransfusie programma verbetert de overleving niet en vermindert de kans op recidief niet bij patiënten met een colorectaal carcinoom.
2. Hoewel een reductie van blootstelling aan allogeen bloed voordelig is, is er geen oncologische reden om een preoperatief autoloog bloeddonatie programma te gebruiken in patiënten met een colorectaal carcinoom.
3. Het verband tussen bloedtransfusies en prognose van het colorectale carcinoom is hetzelfde voor allogeen als voor autoloog bloed.
4. De kans op lokaal recidief is gerelateerd aan de noodzaak om te transfunderen, terwijl dit niet geldt voor de kans op afstandsmetastasen.

5. Niet de bloedtransfusies zelf maar de omstandigheden die transfusies nodig maken bepalen de prognose bij patiënten met een colorectaal carcinoom.
6. Een preoperatief autoloog bloeddonatie programma verlaagt de incidentie van infectieuze complicaties na colorectale chirurgie niet.
7. Indien meer dan 2 eenheden bloed nodig zijn is de kans op infectieuze complicaties vergroot, onafhankelijk of de eerste 2 transfusies allogeen of autoloog waren.
8. Hoewel bloeddonatie de NK cel activiteit verlaagt, heeft deze verlaging geen klinische betekenis voor de prognose en de incidentie van postoperatieve complicaties bij patiënten met een colorectaal carcinoom.

Dankwoord

Graag wil ik hier een kort woord van dank richten aan hen zonder wie dit proefschrift nooit tot stand gekomen was.
Allereerst mijn ouders die mij altijd gestimuleerd hebben om naast mijn opleiding extra activiteiten te verrichten.
Tessa die, mede door erfelijke factoren, het volste begrip heeft kunnen opbrengen voor de repercussies die promoveren voor ons privé-leven had.
Mijn promotor, Prof.Dr. J. Jeekel, die de initiator is geweest van de 'Autologe Bloedtransfusie Trial' en met veel betrokkenheid mij met deze promotie heeft begeleid.
Mijn co-promotor, Richard Marquet, die mij geïntroduceerd heeft in de wetenschap en die altijd bereid was een terneergeslagen onderzoeker weer op te peppen.
De leden van de promotiecommissie die bereid waren om dit proefschrift te beoordelen.
De 'Autologe Bloedtransfusie Trial' welke in dit proefschrift beschreven is, kon alleen voltooid worden door de actieve deelname van de participerende ziekenhuizen en bloedbanken.
Tevens is hier een woord van respect verschuldigd voor Marlene Hoynck van Papendrecht die deze trial vele jaren heeft gecoördineerd en voor Wim Hop die de studie met grote betrokkenheid statistisch heeft begeleid.
De medewerkers van het laboratorium voor experimentele chirurgie en van de afdeling heelkunde van het Dijkzigt ziekenhuis wil ik bedanken voor de plezierige samenwerking waarvan ik vele jaren heb mogen genieten.
De paranimfen Geert Kazemier en Harold Lont dank ik alvast voor de steun tijdens en na de promotie.
Als laatste zou ik diegenen, die ik hier vergeten ben te noemen, willen bedanken voor hun begrip dat er geen proefschrift bestaat dat volledig is.

List of abbreviations

AIDS	acquired immune deficiency syndrome
allo	allogeneic
auto	autologous
Ci	Curie
cm	centimeter
cpm	count per minute
Cr	chrome
FFP	fresh-frozen plasma
FITC	fluorocein isothiocyanate, green fluorescence
g/dl	gram per deciliter
HLA	human leukocyte antigen
KLH	keyhole-limpet hemocyanin
mmol/l	millimol per liter
ml	milliliter
MR	maximal release
NK cell	natural killer cell
n.s.	not significant
PE	phycoerythrin, red fluorescence
PHA	phytohemagglutinin
SD	standard deviation
SEM	standard error of the mean
SR	spontaneous release
µl	microliter

Hemoglobin level of 1.0 g/dl = 0.62 mmol/l
Hemoglobin level of 1.0 mmol/l = 1.61 g/dl

List of publications

-- Busch ORC, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Experimental and clinical results of perioperative treatment with rHuEPO. Pagel H, Weiss C, Jelkmann W (eds). Pathophysiology and pharmacology of erythropoietin, Springer-Verlag 1992; 315-320.

-- Busch ORC, Hop WCJ, Hoynck van Papendrecht MAW, Marquet RL, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 328: 1372-1376.

-- Busch ORC, Marquet RL, Hop WCJ, Jeekel J. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993; 329: 1355-1356 (letter).

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Prognostic impact of blood transfusions on disease-free survival in colorectal carcinoma. Scand J Gastroenterol 1993; 28: Suppl 200: 21-23.

-- Busch ORC, Marquet RL, Hop WCJ, Jeekel J. Colorectal cancer recurrence and perioperative blood transfusions: a critical reappraisal. Semin Surg Oncol 1994; 10: 195-199.

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Autologous blood and infections after colorectal surgery. Lancet 1994; 343: 668-669 (letter).

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Randomized clinical trial comparing the effect of allogeneic and autologous blood in colorectal cancer patients. Houbiers JGA (ed). Blood transfusion in cancer surgery, 1994; 85-97.

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Blood transfusions and local tumor recurrence in colorectal cancer: evidence of a noncausal relationship. Ann Surg 1994; 220: 791-797.

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Blood transfusions and local tumor recurrence in colorectal cancer. Ann Surg 1995; (letter). in press.

-- Busch ORC, Marquet RL, Jeekel J. Allogeneic blood transfusions and cancer prognosis: facts and fiction. Erythropoiesis 1995; 6: in press.

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. The effect of blood transfusions on survival after surgery for colorectal cancer. Eur J Cancer 1995; in press.

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Blood transfusions and postoperative infectious complications after colorectal cancer surgery. submitted.

-- Busch ORC, Hop WCJ, Marquet RL, Jeekel J. Autologous blood transfusions and prognosis in colorectal cancer surgery. J Clin Oncol (letter). submitted.

-- Busch ORC, Hop WCJ, Marquet RL, Lont HE, Jeekel J. The effect of blood donation on prognosis and infectious complications after colorectal cancer surgery. submitted.

-- Hoynck van Papendrecht MAW, Busch ORC, Jeekel J, Marquet RL. The influence of blood loss on tumour growth; Effect and mechanism in an experimental model. Neth J Surg 1991; 43: 85-88.

-- Hoynck van Papendrecht MAW, Jeekel J, Busch ORC, Marquet RL. Efficacy of recombinant erythropoietin for stimulating erythropoiesis after blood loss and surgery; An experimental study in rats. Eur J Surg 1992; 158: 83-87.
-- Marquet RL, Hoynck van Papendrecht MA, Busch ORC, Jeekel J. Blood donation leads to decrease in natural killer cell activity: a study in normal blood donors and cancer patients. Transfusion 1993; 33: 368-373.
-- Marquet RL, Hoynck van Papendrecht MAW, Busch ORC, Jeekel J. Immunological consequences of blood donation. Kretschmer V, Stangel W, Eckstein R (eds). Transfusionsmedizin 1992/93, Karger, 1993; 31: 179-185.
-- Marquet RL, Hoynck van Papendrecht MA, Busch ORC, Jeekel J. R-HuEPO treatment to stimulate erythropoiesis after blood loss and surgery: a dose-finding and efficacy study in rats. Erythropoiesis 1993; 4: 16-21.
-- Marquet RL, Busch ORC, Jeekel J. Autologous blood donation and r-HuEPO therapy to avoid allogeneic blood transfusions in cancer surgery. Erythropoiesis 1994; 5: 77-81.
-- Marquet RL, Busch ORC, Singh SK, Hoynck van Papendrecht MAW, Jeekel J. The influence of anesthesia, surgery, blood loss and blood transfusions on immunity and tumor growth in an animal model. Houbiers JGA (ed). Blood transfusion in cancer surgery, 1994; 49-62.
-- Houbiers JGA, Busch ORC, van de Watering LMG, Brand A, Marquet RL, Jeekel J, van de Velde CJH. Bloedtransfusies in de oncologische chirurgie. Ned Tijdschr Heelk 1994; 4: 145-147.
-- Houbiers JGA, Busch ORC, van de Watering LMG, Marquet RL, Brand A, Jeekel J, van de Velde CJH. Report on a round table meeting and a symposium. Houbiers JGA (ed). Blood transfusion in cancer surgery, 1994; 147-157.
-- Houbiers JGA, Busch ORC, van de Watering LMG, Marquet RL, Brand A, Jeekel J, van de Velde CJH. Blood transfusion in cancer surgery. Eur J Surg 1994; in press.

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