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Stem Cell Transplantation

Heparin-based anticoagulation during peripheral blood stem cell collection may increase the CD34+ cell yield

Heparin combined with acid citrate dextrose (ACD) has been used in children as anticoagulation to diminish secondary effects during leukapheresis. We have found that those procedures performed with heparin and ACD yielded higher numbers of CD34+ cells than those in which the children were anticoagulated only with citrate. The biological explanation of this finding could be found in the events underlying mobilization.

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Heparin is added to the anticoagulation solution in children undergoing leukepheresis to decrease the acid citrate dextrose (ACD) infusion ratio and diminish secondary effects related to citrate chelation of calcium.[1] To the best of our knowledge the influence of the anticoagulation schedule on leukapheresis yield has not been previously studied. We decided to analyze this variable because several recent papers have described some influence of glycosaminoglycan-derived oligosaccharides on the stromal cell-derived factor 1 (SDF-1)-dependent chemotactic effect on peripheral blood progenitor cells (PBPC).[2]

We retrospectively analyzed 257 procedures performed in 224 children diagnosed with a hematologic malignancy (n=88) or solid tumor (n=136), and 33 healthy donors who underwent their first PBPC collection in our unit over the last 10 years. We analyzed the characteristics of all these children, and the procedures (Table 1) in order to evaluate the influence of each variable on the yield. Priming was performed with different cytokines-based schedules (granulocyte colony-stimulating factor, G-CSF; granulocyte-macrophage colony-stimulating factor, GM-CSF). Based on previous studies performed by our group we divided the various schedules into three groups: A (n=32) donors (G-CSF 10 μg/kg/day or G-CSF 5 μg/kg/12 hours subcutaneously), B (n=119). Regular doses of G-CSF (12 μg/kg/day or G-CSF 12 μg/kg/day plus GM-CSF 5 μg/kg/day), and C (n=106); high doses of G-CSF 12 μg/kg/12 hours or 10 μg/kg/12 hours.[3,4] Leukapheresis was performed by the oncology nursing staff on the fifth day of priming as previously described.[5] Initially ACD was the only anticoagulation (ratio between 1:12 to 1:15). Children usually received oral or intravenous calcium supplementation. Since February 1998, in accordance with the experience of Prather *et al.*, anticoagulation with heparin and citrate infused at a higher ratio (30:1) was used.[6] For this solution 5000 units of heparin were added to 500 mL of ACD.

Variables related to the yield were analyzed as previously reported.[3] Data on CD34+ cell count before the apheresis were available in 171 cases so recruitment (ratio of the total CD34+ cells collected to the amount of CD34+ cells estimated to be in peripheral blood prior to the procedure) was analyzed only in these cases.

One hundred and sixty-one children (63%) underwent leukapheresis using anticoagulation with ACD and heparin, as described above, and ninety-six only with ACD. The general leukapheresis characteristics in these two groups were not different (Table 1). PBPC yield did differ between the two groups, as shown in Table 2. Several variables were related to the yield in the multivariate study: prior chemotherapy, previous radiotherapy, child's body weight, and platelet count before the procedure (*data not shown*). Children anticoagulated with heparin plus ACD at a 30:1 ratio had a 2.46 times higher probability of achieving the target CD34+ cell dose than did those anticoagulated with the higher dose of citrate alone ($p = 0.011$-stepwise logistic regression model).

Data on adverse events related to citrate administration were available in 231 patients. Of the children anticoagulated only with ACD, 15 (20%) suffered secondary effects of citrate infusion whereas six children (4%) did so in the other group ($p < 0.0001$). However, adverse hemorragic events were more frequent in the latter group: hematoma occurred at the vascular access site in 1 ACD-anticoagulated patient but in 9 anticoagulated with ACD and heparin and mild hemorrhage after catheter was removed and occurred in 8 patients anticoagulated with heparin plus ACD but in none anticoagulated only with ACD). Bleeding was controlled with local measures. Recruitment was adequate in 111 cases (65%). However, only 25% (3/12) of patient anticoagulated with ACD had

Table 1. Leukapheresis characteristics.

Characteristics	*ACD ratio 1:12 to 1:15 (n=96)*	*ACD and heparin at a ratio of 1:30 (n=161)*	*Unpaired t-test*
Age (years), median (range)	7 (1-18)	7 (1-18)	N.S.
Weight (kg), median (range)	25 (7-63)	26 (5-109)	N.S.
Donation status donors/patients	9/87	24/137	N.S.
Total blood volume processed (L) median (range)	4.5 (1.6–8.8)	4.4 (1.7-12.3)	N.S.
Blood volume processed (mL) median (range)	9188 (2209-25375)	9102 (1942-22987)	N.S.
Blood volume processed per kg body weight (mL) median (range)	344 (117-670)	331 (136-804)	N.S.
Children who achieved the target $CD34^+$ cell count of $>2\times10^6$/kg (%)	62.5%	78.3%	$p<0.01$
Pre-apheresis leukocyte count ($\times10^6$/mL) median (range)	36 (4.6-142)	42 (7.9-152)	N.S.

Table 2. $CD34^+$ cell yield.

	Total (n=257) Median (range)	*Anticoagulation with ACD-A Median (range)*	*Anticoagulation with ACD-A plus heparin Median (range)*	*p analyzed by the Mann-Whitney test*
Total $CD34^+$ cells collected $\times10^8$	1.14 (0.06-11.12)	0.83 (0.006-11.1)	1.36 (0.07-8.97)	0.006
$CD34^+$ cells/kg BW $\times10^6$ harvest	4.4 (0.01-53.1)	2.75 (0.01-50.9)	4.9 (0.1-53.1)	0.0054
$CD34^+$ cells/kg BW/Lbvp* $\times10^6$	0.45 (0.01-22.5)	0.33 (0.01-12.7)	0.51 (0.01-22.5)	0.02

**Lbvp: liter of blood volume processed.*

recruitment versus 68% (108/159) of those in whom heparin was used ($p<0.005$). In the multivariate analysis, higher blood volume processed (OR: 1.08; 1.004-1.01; $p<0.0001$), duration of the leukapheresis (OR: 1.007; 1.001-1.014; $p<0.05$), lower leukocyte count before the procedure (OR: 0.995; 0.96-0.99; $p<0.005$), and anticoagulation with heparin and ACD (OR: 10.47; 2.12-51.77; $p<0.005$) were variables positively associated with achieving recruitment.

Prior chemotherapy, previous radiotherapy, the children's body weight, and platelet count before the procedure are related to the outcome of the collection.[3,4,7] The anticoagulation schedule has also been found to be related to the yield. The explanation for this finding could lie in the greater availability of $CD34^+$ cells in procedures in which heparin was used, thus achieving recruitment more frequently than leukapheresis in which heparin was not used (68% vs 25%). The heparin and other glycosaminoglycan binding properties of stromal-cell derived factor (SDF-1α) are well recognized.[8,9] On the other hand, Sbaa-Ketata *et al.* have recently studied the role of different glycosaminoglycan (hyaluronan) fragments on SDF-1α dependent chemotaxis on $CD34^+$ cells.[2] These authors described how the chemotaxis effects of SDF-1α on progenitor cells were enhanced by adding different glycosaminoglycan-derived oligosacharides to an *in vitro* chemotactic system.[2] The role of heparin or its different catabolized fragments on the mobilization of progenitor cells needs to be clarified in the future, but it seems that some of these fragments could modify progenitor cell trafficking leading to an increased number of these cells in peripheral blood during leukapheresis.

However, this finding should be considered with caution, given the retrospective nature of the study. Randomized prospective studies are needed to clarify the role of anticoagulation on progenitor cell yield and whether this heparin-containing schedule should be used regularly to enhance collection.

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Infectious Diseases

Decrease of dual hepatitis B and C virus infections in children with cancer: changes in risk factors over 30 years

The frequency of dual hepatitis C and B virus infections and the impact of risk factors were evaluated in a cohort of 420 children with cancer. Multivariate analysis showed that primary cancer diagnosis, duration of therapy, and specific immunoprophylaxis were significant variables influencing the incidence of dual viral hepatitis, whereas other risk factors had no impact in this group.

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Children with cancer are a group at high risk of dual hepatitis B (HBV) and C (HCV) infection.[1-3] For these children, the major risk factors for acquiring viral hepatitis have been reported to be endemic environment, blood transfusions, major surgical procedures, infection in the family, and frequent medical procedures connected with percutaneous or mucosal invasion.[4-7]

HBV and HCV are the main causes of chronic viral hepatitis and chronic liver disease in children with cancer.[8-9] We analyzed two groups of Polish children with cancer treated in different time periods when different preventive procedures for viral hepatitis infections were available.

A total number of 420 children with cancer were included in the study. All children were treated in one center, followed-up for at least 6 months, and tested for HBV and HCV serum and/or liver specimen markers, including HCV-RNA by RT-PCR. The patients were divided into 2 groups, with regard to period of beginning anticancer therapy and strategy of general prophylaxis (Table 1).

The general strategy to control HBV and HCV infections involved a multi-step procedure, including initiation of blood donor screening for HCV infection; institution of anti-hepatitis B immunization;[10] intensification of universal precautions as standard of care; administration of an educational program for staff, patients, and families; and acquisition of contemporary blood collection equipment. The introduction of this prophylaxis was completed in 1996.

In group I, patients treated before 1997, 110 (44.2%) children had no markers of infections, 53 (21.3%) were infected with HCV only, 33 (13.2%) with HBV only and 53 (21.3%) had dual infection. The cumulative risk of dual infection for all patients was 44.7% (Figure 1A). Patients with leukemia were at 2.5-fold (95%CI=0.99-6.90, p = 0.032) and 5-fold (95%CI = 1.89-14.03, p = 0.0002) higher risks of dual infection than those with lymphomas and solid tumors, respectively. The risk of dual infection was 2.6-fold higher for blood recipients (95%CI = 1.02-7.44, p = 0.028), however the number of units of blood transfused had no impact on the frequency of any infections. There was no influence of surgical treatment on incidence of dual infection, or on any single infection. In children who were included in an immunoprophylaxis program against HBV infection, the risk of chronic HBV and chronic dual infection was 8.1-fold (95%CI=2.01-33.3, p = 0.0001) and 2.8-fold (95%CI=1.41-5.61, p = 0.0001) lower, respectively, than in the other children. In Cox univariate analysis, development of chronic dual hepatitis B and C was related to mean alanine transferase (ALT) value (p = 0.0389), blood transfusions (p = 0.0281), duration of anticancer therapy (p = 0.0011), primary cancer diagnosis (p = 0.0796) and anti-HBV immunoprophylaxis (p = 0.0020), but not to age, gender, living place, mean and peak serum bilirubin level, having undergone surgical procedures, the number of units of blood transfused prior to infection, and infections among relatives. In Cox multivariate analysis, anti-HBV immunoprophylaxis (p = 0.0002), duration of anticancer therapy (p = 0.0092), and mean ALT activity (p = 0.0001) were the only factors significantly related to development of dual hepatitis. The median ALT activity in the dual HBV/HCV group was higher than in all other patients taken together (121 vs 78 IU/L, p < 0.0001). There were no differences in median bilirubin level between the HBV/HCV group and the other patients.

In group II, the patients treated after 1997, 149 (87.1%) children had no markers of viral infection; 10 (5.85%) were infected with HBV only, including 3 with HBV infection acquired before the anti-cancer therapy; 10 (5.85%) with HCV only, and 2 (1.2%) patients had dual infection. The incidence of dual HBV/HCV infections decreased very significantly from 53/249 in group I to 2/171 group II (p = 0.0000)

Table 1. Main characteristics of the study population.*

	Group I	*Group II*
Period of inclusion	1974-1997	1997-2003
Number of patients	249 (53)	171 (2)
Gender male:female	148:101	91:80
Mean age at diagnosis 95% CI (years)	7.7/3.2-12.2	7.2/2.3-12.1
Mean follow-up 95% CI (months)	189/168-210	37/19-55
HBV immunoprophylaxis	184 (30)	171 (2)
Diagnosis		
Leukemias	129 (40)	73 (1)
Lymphomas	47 (7)	22 (0)
Solid tumors	73 (6)	76 (1)

**The number in brackets indicates the number of patients with dual HBV/HCV infection.*