To the editor:

Cytomegalovirus infection and disease after autologous CD34-selected peripheral blood stem cell transplantation for multiple myeloma: no evidence of increased incidence based on polymerase-chain-reaction monitoring

Recently, Holmberg et al reported an increased incidence of cytomegalovirus (CMV) disease following autologous CD34-selected peripheral blood stem cell transplantation (PBSCT).[1] We have also been interested in the possibility of delayed immune reconstitution following autologous CD34-selected PBSCT, especially after the development of an EBV-lymphoma in one of our patients.[2] We have performed 44 CD34-selected autologous PBSCT procedures at our institute in patients with plasma cell dyscrasias (42 multiple myeloma, 1 relapsed solitary plasmacytoma, 1 POEMS syndrome). In 13 cases the patient was CMV seronegative, and none had evidence of CMV disease after transplantation. Details of the 31 transplantation procedures in CMV seropositive patients are shown in Table 1. In our experience, the absolute T-cell count ($\times 10^5$ cells) in the CD34-purified fraction from the 3 different devices used for selection was 72 (54-184), 25 (10-184), and 0.3 (0.2-31) for the CellPro Ceprate, Baxter Isolex 300i, and Miltenyi CliniMacs, respectively, corresponding to 3, 3.6, and 5.7 logs of T-cell depletion.[3] Patients routinely received low-dose prophylactic aciclovir but not intravenous immunoglobulin (IVIG). We have seen only one case of CMV disease (interstitial pneumonia) in these patients. This case was treated successfully with ganciclovir and IVIG. The study by Holmberg et al suggested that the use of lower doses of $CD34^+$ cells with a TBI-based conditioning regimen increased the risk of development of CMV disease. The lower rate of CMV disease (3.2%) in our study compared to that of Holmberg et al (22.6%) cannot be explained by differences in these parameters, because we administered lower doses of CD34-positive cells (median 2.8×10^6/kg versus 4.8×10^6/kg) and more of our patients received TBI-based conditioning regimens (71% versus 45.2%). Furthermore, all of our patients also had an underlying diagnosis of plasma cell dyscrasia, a subgroup noted to have an increased risk for the development of CMV disease by Holmberg et al (4 of their 6 patients with multiple myeloma developed CMV disease).

Table 1. Patient characteristics

Number of patients	31
Age (median, range)	54 (40-66)
Gender	
Male	21
Female	10
Prior chemotherapy	
Number of regimens (median, range)	1 (1-2)
Prior radiotherapy (local)	8
Selection device	
Ceprate	24
CliniMACS	6
Isolex	1
CD34 dose ($\times 10^6$ cells/kg) (median, range)	2.8 (0.7-14.2)
Conditioning	
TBI/melphalan	22
Melphalan	9

Table 2. Results of CMV PCR surveillance in CMV seropositive patients

CD34 selection device	Patient number	Number of PCR positive patients	Number receiving ganciclovir
Ceprate	7	3	1
CliniMACS	5	2	0
Unselected	5	3	0

Of the CMV seropositive patients, 12 were monitored prospectively for CMV infection by whole blood polymerase chain reaction (PCR). Surveillance was performed weekly while they were inpatients; then it was performed at outpatient follow-up until 3 months after transplantation. Results up to 3 months are also available on 5 unselected procedures performed on patients with multiple myeloma. The results are shown in Table 2. Although the numbers are small, there is no apparent difference in the rates of CMV PCR positivity between the selected patients (5 of 12) and the unselected patients (3 of 5). These rates of CMV positivity are similar to those previously reported in unselected autologous bone marrow or PBSC transplants (42.2%).[4] Only one patient had more than 2 positive results on consecutive weeks. The positive results were coincident with ''fever of unknown cause.'' The patient did not have bronchoalveolar lavage performed because of the lack of respiratory symptoms but was treated with ganciclovir with resolution of the pyrexia. All patients with a single documented positive result remained asymptomatic and received no treatment.

We have therefore found that a single CMV PCR result has a low positive predictive value for the development of CMV disease in CD34-selected PBSC transplants. Quantitative PCR may be a more informative test, although to date there are no published studies using this technique in the setting of CD34 selection. The negative predictive value of the test is of interest in view of the finding of Holmberg et al that 2 of the 3 patients who developed CMV pneumonia in the pp65 antigenaemia screened group did not have preceding CMV antigenaemia.[1] CMV PCR has been reported to have a negative predictive value of 100% in unselected autologous bone marrow and PBSC transplants.[4] Although we have not seen any cases of CMV disease without preceding PCR positivity, the low rate of CMV disease in our population does not allow us to comment further on the negative predictive value in the setting of CD34 selection. In summary, we have not seen an increase in CMV disease in patients with multiple myeloma after autologous CD34-selected PBSC transplantation despite some of our patients receiving apheresis products with up to 2 logs greater T-cell depletion than achieved with the Ceprate/Isolex selection devices used in the recent study of Holmberg et al.

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Response:

Increased incidence of cytomegalovirus infection and disease after autologous CD34-selected PBSC transplantation

Unlike the work of Peggs et al, our paper reported on the incidence of cytomegalovirus (CMV) disease and infection in CMV seropositive patients with a number of different diseases who received a transplant with autologous CD34-selected peripheral blood stem cells (PBSCs).[1] Overall, 7 out of 31 (22.6%) CD34-selected patients developed CMV disease within 100 days after transplantation. In a univariate analysis, CD34 selection alone was significant for the development of CMV disease, with an odds ratio (OR) of 6.62 (confidence interval [CI], 2.3-19; $P < .001$). By evaluating combinations of factors together in a multivariate logistic regression model, the inclusion of conditioning with a TBI-based regimen and the dose of CD34 cells infused amplified the effect of CD34 selection. With respect to the development of CMV infection, CD34 selection and steroid use were highly significant in a univariate analysis: OR was equal to 3.0 and 2.69, respectively. Because all patients in our CD34-selected group and 3 out of 10 patients in the unselected group who developed CMV disease had received a transplant for multiple myeloma or lymphoma, an additional subset univariate analysis was performed. In this analysis, CD34-selected patients had a significant chance of developing disease, with an OR of 17, (CI, 3.8-76.7; $P < .001$).

We are not the only group to report an increased incidence of CMV disease associated with CD34 selection. Recently, Stocherl-Goldstein et al[2] presented their results. In 40 multiple myloma patients who received CD34-selected cells, there were 4 (10%) patients who developed CMV disease.

In our cohort of patients, there were 6 patients with multiple myeloma who received CD34-selected cells and 26 who did not. Four of the 6 CD34-selected patients developed CMV disease, as compared to 1 of the 26 unselected.

As mentioned in the discussion section of our paper, we believe that differences in the reported incidence of CMV disease are due to differences not only in the evaluation of patients but in the immunologic function of the patients at risk. In our 6 multiple myeloma patients who received CD34-selected PBSC, all were mobilized with intermediate dose chemotherapy for stem cell collection. The number of regimens the patients received prior to their mobilization chemotherapy were 1, 2, 2, 2, 2 and 3, thus making our multiple myeloma patients probably more heavily treated than the group treated by Peggs et al, which received a median of 1 chemotherapy regimen. In addition, 3 of our 6 CD34-selected multiple myeloma patients received steroid therapy after transplantation. In general, we have found the following differences in the median number of immune cells infused in the CD34-selected and unselected stem cells product: CD3, 8.8 vs 1273; CD4, 4.2 vs 518; CD8, 6 vs 246; $CD3^+/CD56^-$, 10.8 vs 865; $CD3^+/CD56^+$, 0.79 vs 123; and $CD3^-/CD56^+$, 2.55 vs 158×10^6. Patients who developed CMV disease after the infusion of CD34-selected cells had the lowest number of immune cells infused, with a median of 2.5 CD3, 3.1 CD4, 4.6 CD8, 7.6 $CD3^+/CD56^-$, 0.49 $CD3^+/CD56^+$, and 2.2×10^6 $CD3^-/CD56^+$. At present, we are in the process of retrospectively determining the number of specific T cells that recognize CMV that were infused in the different patient populations.

We agree with Peggs et al that CMV antigenemia is not helpful, because 2 of 3 of our patients who developed CMV disease had no evidence of CMV antigenemia. In addition, the median day to developing disease among our CD34-selected patients was 26 days (range, 16-76 days). Thus the early onset of CMV disease may make it hard to follow patients by CMV antigenemia testing because their white blood cell count may be too low. We also agree with Peggs et al that PCR by itself may not be the best monitoring for risk for CMV infection and disease and that quantitative PCR may be more informative. To date, because there are no published studies using quantitative PCR in the setting of CD34 selection, we have adopted a policy at our institution of screening all patients prior to initiation of transplant conditioning with a quantitative PCR. All patients who have at least 100 copies of CMV in their blood are treated with prophylactic antiviral therapy during conditioning. Weekly after the infusion of CD34-selected PBSC, all patients are screened also by CMV PCR. A level of at least 100 copies of CMV results in the initiation of antiviral therapy.

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To the editor:

Myocardial ischemia following allogeneic bone marrow transplantation: possible implication of tacrolimus overdose

It has been reported that clinically significant heart involvement affected 5% to 10% of patients undergoing bone marrow transplantation (BMT) after pretreatment with CY and TBI[1] and that life-threatening or fatal cardiac toxicity occurred in about 1%-5%