risk of 18.6% at 5 years in 86 follicular lymphoma patients autografted in first response.[2] In our study, 23 patients out of 35 (65%) responded to an initial anthracycline-based chemotherapy (CHOP or CHOP-like) while 7 patients needed salvage treatment with high doses of cytarabine before ASCT and 5 patients never obtained a response good enough for ASCT. This proportion of MCL patients responding to an anthracycline-based chemotherapy is consistent with other results reported in the literature.[3] Rituximab is likely to play an important role in association with anthracycline-based chemotherapy by effectively clearing blood and bone marrow lymphoma cells.[4] However, the observation that addition of rituximab to induction therapy does not translate into prolonged progression-free survival supports the role of using ASCT in first response.[5,6]

In a recent landmark study, Dreyling *et al*.[7] demonstrated that ASCT prolongs progression-free survival in MCL. They reported 3-year overall survival and progression-free survival rates of 83% and 54%, respectively. The corresponding 5-year rates in our study, dealing with a comparable population of patients, were 62% and 40%, respectively, thus confirming after an extended follow-up that ASCT in first response is an effective and safe treatment for MCL patients under 65 years of age.

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Stem Cell Transplantation

Development of functional *Haemophilus Influenzae* type b antibodies after vaccination of autologous stem cell transplant recipients

Sixteen autologous stem cell transplant recipients received three vaccinations with conjugated *haemophilus influenzae* type b vaccine. Quantitative and qualitative aspects of the antibody response were studied. The vaccination schedule resulted in high antibody response rates and functional maturation of antibodies, as measured by antibody avidity and phagocytosis-inducing capacity.

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Infections are a major source of morbidity in patients undergoing autologous stem cell transplantation and are frequently caused by encapsulated bacteria such as *Haemophilus influenzae* type b (*Hib*) and *Streptococcus pneumoniae*.[1,2] Therefore, vaccination of stem cell transplant recipients with *Hib* and pneumococcal vaccine has been recommended.[3,4] A response to vaccination is often quantitatively expressed as antibody titers, but determination of avidity and phagocytosis-inducing capacity of antibodies can provide important information regarding the functional activity of antibodies.[5,6] For instance, an increase in antibody avidity during the year following *Hib* vaccination with a concurrent decrease in antibody levels, has been described in children.[7] We conducted a prospective follow-up study to determine quantitative and qualitative aspects of the humoral immune response to multiple vaccinations with conjugated *H. influenzae* type b vaccine in 16 adult patients with non-Hodgkin's lymphoma (n=3) or multiple myeloma (n=13) who underwent autologous stem cell transplantation. Patients with multiple myeloma received high dose melphalan, whereas patients with non-Hodgkin's lymphoma received the BEAM regimen as conditioning therapy. At 6, 8 and 14 months after transplantation, patients were vaccinated with *Hib* (PRP-T vaccine: polyribosylribitolphosphate conjugated to tetanus toxoid). Serum samples were taken before vaccination and 3 weeks after each vaccination. For each patient, sera taken at all time points were analyzed simultaneously for all techniques. IgG antibody levels to *H. influenzae* were measured by ELISA as described previously.[8] An adequate antibody response was defined as a 4-fold or greater increase in antibody levels in addition to a minimal titer of 50 U/mL corresponding to 18.8 µg/mL, which is 50% of the titer in the reference serum. Avidity indices of IgG anti-*Hib* antibodies were measured by a modification of the sodium thiocyanate (NaSCN) elution method described by Pullen *et al*.[9] Antibody avidity can only reliably be determined in sera with a minimal optical density value of 1.0 at a 1:50 dilution, corresponding to a minimal *Hib* antibody concentration of 25 µg/mL. The relative

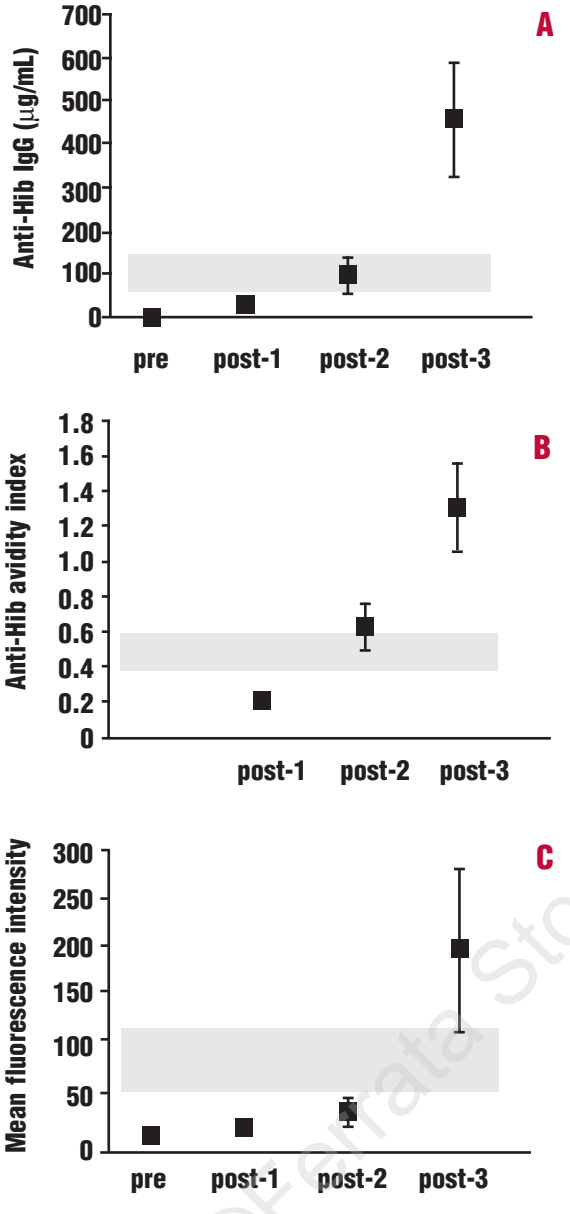

Figure 1. A. Anti-*Hib* IgG antibodies of 14 patients before (pre) and after one (post-1), two (post-2) and three (post-3) vaccinations with conjugated *Hib* vaccine. Mean antibody levels plus standard errors are shown. Anti-*Hib* antibodies increased significantly after three vaccinations (p value=0.001). Hatched area indicates mean value (± SE) of 12 healthy adults vaccinated with a single dose of *Hib* conjugate vaccine. B. Anti-*Hib* IgG antibodies avidity indices (AI) after one (post-1), two (post-2) and three (post-3) vaccinations with *Hib* vaccine. Mean AI plus standard errors are shown. Mean AI increased significantly after three vaccinations with conjugated *Hib* vaccine, as compared with AI after two vaccinations (p value=0.047). Hatched area indicates mean value (± SE) of 12 healthy adults vaccinated with a single dose of *Hib* conjugate vaccine. C. Phagocytosis inducing capacity of anti-*Hib* IgG antibodies of 14 patients, expressed as mean fluorescence intensity (MFI), before and after one, two and three vaccinations with *Hib* vaccine. MFI plus standard errors are shown. MFI increased significantly after two and three vaccinations, as compared with MFI before vaccination (p values 0.03 and 0.002, respectively). Hatched area indicates mean value (± SE) of 12 healthy adults vaccinated with a single dose of *Hib* conjugate vaccine.

avidity index (AI) is defined as the molarity of NaSCN at which 50% of the amount of IgG antibodies bound to the coated antigen in the absence of NaSCN has been eluted. Phagocytosis-inducing capacity of anti-*Hib* IgG antibodies was determined by a modification of the method described by Sanders *et al.*[10] In brief, sera of patients were incubated with fluoroscein isothiocyanate (FITC)-labeled *Hib*, subsequently incubated with polymorphic mononuclear cells and analyzed by flow cytometry. The phagocytosis-inducing capacity of antibodies was expressed as the mean FITC fluorescence intensity (MFI).

The mean level of $CD19^+$ cells before vaccination was 0.17×10^9/L (range 0-0.4×10^9/L) with subnormal levels of B cells in three patients. Two patients had recurrence of tumor at 9 and 12 months after transplantation and were not included in the subsequent analysis. Measurement of antibody titers showed response rates of 38%, 75% and 93% after one, two and three vaccinations, respectively (Figure 1A). One patient did not acquire anti-*Hib* antibodies after any of the three vaccinations. Avidity indices are depicted in Figure 1B. After repeated vaccinations, avidity of anti-*Hib* IgG antibodies increased in all but one of the patients who were eligible for avidity determination. This one patient did show an increase in anti-*Hib* antibodies after all vaccinations, but after the third vaccination the avidity index decreased.

The phagocytosis-inducing capacity of anti-*Hib* IgG antibodies is shown in Figure 1C. The previously mentioned patient who did not acquire anti-*Hib* antibodies did not show an increase in phagocytosis-inducing capacity. This patient had a very low relative CD19-count in the circulation (<1%) at the time of the first vaccination, also reflected by low serum immunoglobulin levels. The patient who had a high increase in antibodies but a decrease in avidity index, also showed a decrease in MFI after the third vaccination. Although this patient did have a robust IgA response after the third *Hib* vaccination (a rise from 163 to 2034 U/mL) which was higher than that in the other patients (range 1 to 643 U/mL after three *Hib* vaccinations), the high IgA antibody response cannot readily explain the drop in IgG avidity, apart from the theoretical possibility that all high affinity IgG-bearing B lymphocytes would have switched to IgA, resulting in blocking of phagocytosis. However, the data do underline the relation between antibody avidity and opsonization/phagocytosis and show that an increase in antibody quantity is not always accompanied by an increase in antibody quality.

In conclusion, in this study of autologous stem cell transplant recipients, multiple vaccinations with a conjugated *Hib* vaccine resulted in high antibody levels and maturation of antibody functionality.

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