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Absolute Lymphocyte Count Predicts the Response to New Influenza Virus H1N1 Vaccination in Pediatric Cancer Patients

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We measured the vaccination response to the new H1N1 virus in relation to the lymphocyte count prior to vaccination in pediatric cancer patients. The absolute lymphocyte count above the lower normal limits (LNL) for age prior to vaccination predicts the response to influenza vaccination in pediatric cancer patients treated with chemotherapy.

The efficacy of vaccination in (pediatric) cancer patients is still a topic of debate. Most chemotherapeutic treatments lead to an impairment of the cellular immune response, which is the main risk factor for viral infections. These infections can cause severe morbidity in children with cancer (1, 2, 3). In April of 2009, a new swine-origin influenza A H1N1 virus was detected in humans and was subsequently declared as an influenza pandemic by the World Health Organization (WHO) (4). According to the national and international guidelines, vaccination was recommended for immunocompromised children (5). Vaccination against this new influenza A H1N1 virus, which is a neoantigen, allowed us to assess immunologic determinants in pediatric cancer patients that predict a protective response following vaccination. We studied the vaccination response to this new swine-origin influenza A H1N1 virus in pediatric cancer patients in relation with absolute counts of the lymphocytes and its subpopulations.

Children with cancer being treated with chemotherapy, or within 6 months after the end of chemotherapy, were vaccinated twice (3-week interval) with an intramuscular injection with an inactivated split-virion preparation of the A/California/07/2009(H1N1)v-like strain (X-179A), which contained 7.5 μg of hemagglutinin per dose of 0.5 ml. Patients with a known PCR-proven H1N1 influenza infection before the first vaccination were excluded. Blood sampling was done before vaccination and 3 weeks following the second vaccination. Antibody levels specific for A/California/07/2009(H1N1) were determined by serum hemagglutination inhibition (HI) assay before vaccination and 3 weeks after vaccination. A protective response was defined as achieving a HI antibody titer of ≥1:40 following vaccination. Prior to the first influenza vaccination (day −10 to day −1), blood samples were collected from the patients to analyze the total white blood count (TWBC) and the absolute lymphocyte count. Three

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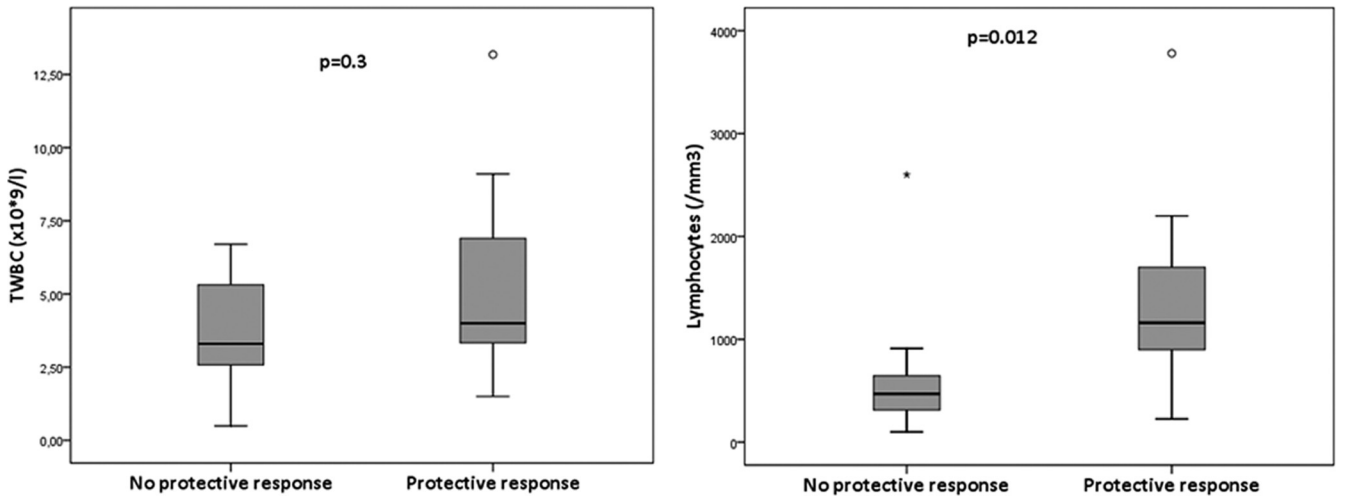

FIG 1 Relation between the total white blood count and the absolute lymphocyte count prior to the first vaccination and the response to vaccination. The relation between the total white blood count and the absolute lymphocyte count was studied using the Mann-Whitney U test.

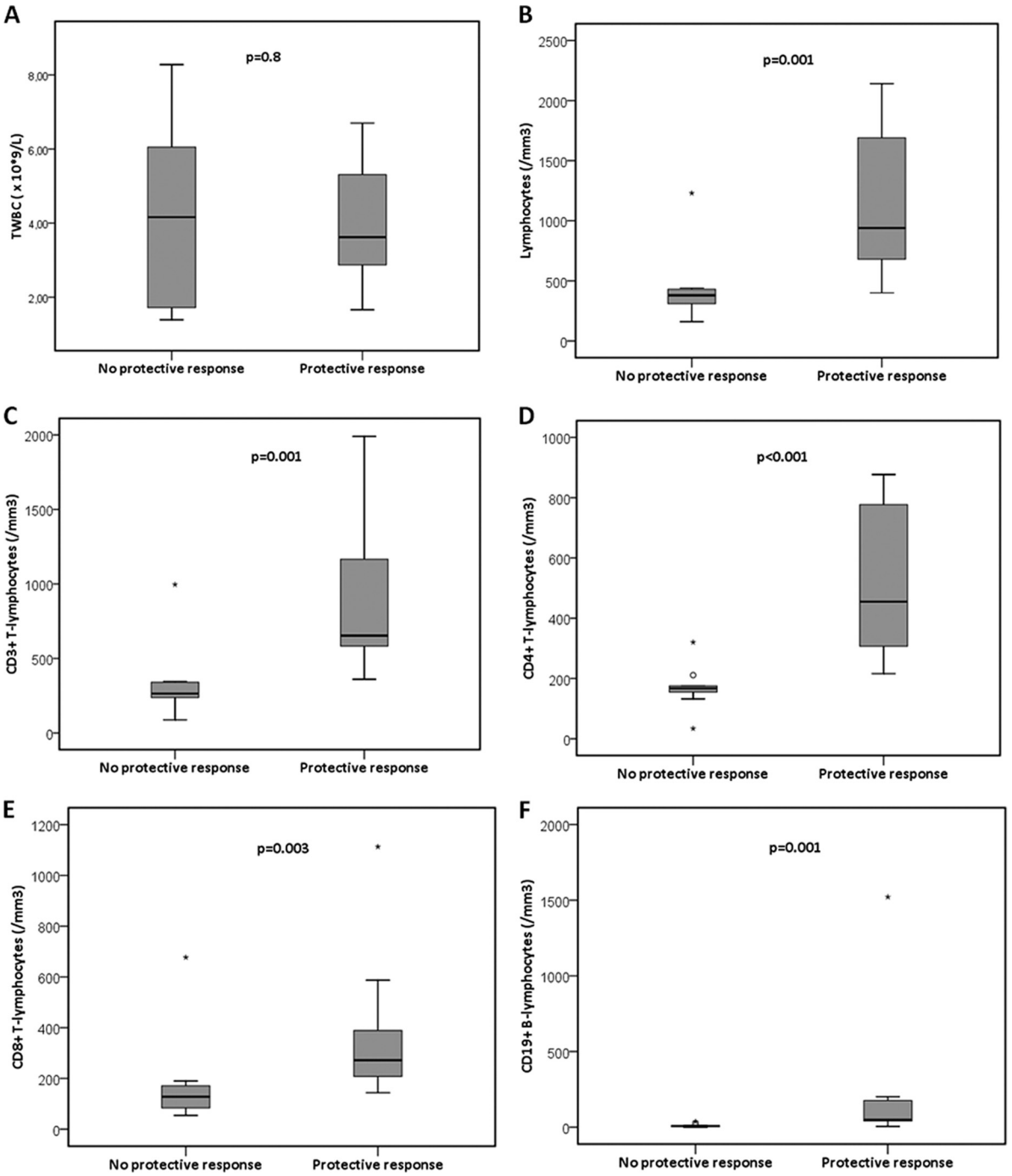

FIG 2 Relation between the total white blood count (A) and the lymphocyte subpopulations (B to F) and the response to vaccination. The relation between the absolute values of the lymphocyte subpopulations was studied using the Mann-Whitney U test.

weeks after vaccination, an additional 3 ml of heparin blood was obtained from a random subset of patients for analysis of lymphocyte subpopulations using standard flow cytometrical immunophenotyping. Lower normal limits (LNL) for the absolute lymphocyte counts according to age were defined as 1,700/mm^3 for age 2 to 4 years, 1,100/mm^3 for age 5 to 9 years, and 1,000/mm^3 for age 10 years to adult (6). A comparison between the vaccination response and categorical factors was studied using the two-sided Fisher exact test. A comparison between the vaccination response and continuous variables was studied using the using the Mann-Whitney U test.

Of the 41 included patients, 2 patients did not receive the vaccinations, and 6 patients did not receive the second vaccination for different reasons. Thirty-three patients were analyzed further (15 female and 18 male; median age, 6 years [range, 2 to 17 years]). The patients suffered from acute lymphoblastic leukemia (ALL) ($n = 19$), acute myeloid leukemia (AML) ($n = 2$), Langerhans cell histiocytosis (LCH) ($n = 1$), atypical teratoid rabdoid tumor

(ATRT) ($n = 1$), medulloblastoma ($n = 3$), neuroblastoma ($n = 1$), non-Hodgkin lymphoma (NHL) ($n = 1$), optical glioma ($n = 3$), rhabdomyosarcoma ($n = 1$), and Wilms' tumor ($n = 1$). Six of the 33 patients were treated with oseltamivir phosphate because of fever of unknown origin, later proven to be H1N1 negative by PCR. Twenty-nine patients were vaccinated during chemotherapy, four patients within 6 months after chemotherapy. Analysis of the immune response was possible in 31 patients. Two patients were excluded from the analysis of the immune response to vaccination: one patient had a HI antibody titer of ≥320 before the first vaccination without clinical symptoms, and one patient developed a PCR-proven H1N1 directly after the first vaccination dose. Of the 31 patients, 20 (65%) showed a rise in titer after vaccination, ranging from 1:20 to 1:640. Of these 31 patients, 18 (58%) had a protective immune response (>1:40). Twenty patients were treated for hematological malignancies, and 11 patients were treated for solid tumors. There was no significant difference in the vaccination response between the two diagnosis groups. We were able to obtain values of the TWBC and the absolute lymphocyte count from 28 patients in the period 10 days to 1 day prior to the first vaccination. The relation between these two parameters and the vaccination response is shown in Fig. 1. There is a significant difference in the absolute lymphocyte count prior to the first vaccination between the patients with a protective versus no protective vaccination response ($P = 0.012$). Absolute lymphocyte counts for above the LNL for age were seen in 13 of 28 patients (46%). In 12 of these 13 patients (92%), a protective response to vaccination was seen. In the 15 patients with absolute lymphocyte counts below the LNL for age, only 5 (33%) had a protective response to vaccination ($P = 0.002$). We were able to obtain samples for lymphocyte subpopulations in 22 patients. In Fig. 2, the relation between the total white blood cell count (TWBC) and the lymphocyte subpopulations and the response to vaccination is shown. There is a significant relation between the response to vaccination and levels of all the different lymphocyte subpopulations ($P < 0.001$ for the $CD4^+$ T cells, and $P = 0.001$ for the other subpopulations) (Fig. 2). We found that if the absolute $CD4^+$ T cell count is less than 200/mm^3, no protective vaccination response can be observed.

Vaccination of immunocompromised pediatric cancer patients with inactivated split-virion preparation of influenza vaccine is considered to be safe (1–3). In our study, 58% of the patients had a protective response to vaccination. This is similar to data on vaccination response in pediatric cancer patients from previous influenza vaccinations (3). In addition, it is also similar to well-controlled HIV-infected patients, who showed a protective response in 60% (7). An important finding of our study was that an absolute lymphocyte count above the lower normal limit for age prior to vaccination led to a protective response to vaccination. This result gives us a predictive tool to determine at what moment vaccination in pediatric cancer patients will be effective. Shahin and colleagues failed to show a significant influence of a prevaccination lymphocyte count (above 1,000/mm^3 versus 1,000/mm^3 or less) on seroresponse rates in children with solid tumors (8). Yen and colleagues obtained results similar to those in our study. They showed that patients with absolute lymphocyte counts less than 1,500/mm^3 during the vaccination period had a nonseroresponse (9). Assessment of absolute counts of lymphocyte subpopulations after vaccination showed that a protective response to vaccination was not observed in patients with an absolute $CD4^+$ T lymphocyte count of less than 200/mm^3. $CD4^+$ T lymphocyte counts were only determined after vaccination, thus it could be possible that $CD4^+$ T lymphocytes were slightly different at the time of vaccination. The crucial role of $CD4^+$ T cells for the generation of an effective antibody response to influenza vaccination has been described previously (7). In HIV-infected individuals, suboptimal influenza vaccine responses with $CD4^+$ T cell counts <200/mm^3 have been reported repeatedly (10). The role of $CD4^+$ T cells for an effective antibody response against the new H1N1 vaccine in HIV-infected individuals has also been reported (11). H1N1 vaccination has been clearly effective in the healthy adult population, with a protective vaccination response in more than 95% (12), as well as in healthy infants and children (92.5%) (13). The best approach to measure immunogenicity to influenza vaccination is a much-debated topic, especially in oncology patients (2). It should be appreciated that hemagglutination (HA) inhibition measures only part of the host response, and it has become clear that the neutralizing and protective antibodies to the H1N1 influenza strain in many patients following natural infection were often not hemagglutination inhibition positive, as they target the hemagglutinin stalk and not the hemagglutinin globular head (14).

In summary, the absolute lymphocyte count above the LNL in pediatric cancer patients predicts a protective response to vaccination. These findings have important implications for the establishment of a response to vaccination in pediatric cancer patients during treatment with chemotherapy.

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