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Case report

Erythroblastaemia in natalizumab-treated patients with multiple sclerosis

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ABSTRACT

Background: Natalizumab is a monoclonal antibody that significantly reduces the occurrence of relapses in relapse-remitting multiple sclerosis (RRMS) patients. Early papers on the clinical use of natalizumab in RRMS patients reported erythroblastemia as occasional and transient.
Objectives: to determine the prevalence and absolute count of erythroblasts (nucleated red blood cells, NRBCs) in peripheral blood of RRMS patients in different treatment groups and healthy controls from the same geographic area using the same equipment for laboratory analysis.
Methods: We retrospectively evaluated the samples of 203 consecutive RRMS patients including 26 subjects on natalizumab, 17 on fingolimod, 72 on interferon, 41 on glatiramer acetate, 47 treatment-naïve and 240 healthy controls from the same geographic area. Blood samples were processed using an XN-9000-Hematology Analyzer and subsequent microscopic verification. In the natalizumab-treated patients we performed an additional analysis in order to detect the expression of CD34+ cells in peripheral blood, as confirmation of a bone marrow mobilization.
Results: The prevalence of patients with NRBCs positivity was significantly higher in natalizumab-treated patients (92%) compared with the other treatment groups and healthy controls (0%) ($p < 0.0005$). The median absolute NRBCs count was significantly higher in natalizumab-treated patients (median 0.020, $p < 0.0005$) than in the other treatment groups and healthy controls. Natalizumab-treated patients also had higher levels of white blood cells than all other groups and lower haemoglobin levels than healthy subjects ($p < 0.01$), but no morphologic alterations were evident at a subsequent review of red blood cells, platelets and white blood cells. CD34+ cells levels were consistent with mobilization of haematopoietic stem cells from the bone marrow (median 8 cells/μL, IQR 5–12).
Conclusions: We confirm erythroblastaemia as a frequent finding of natalizumab treatment in RRMS patients. More extended knowledge and adequate long-term observation of this phenomenon are essential to better understand any pathological implication.

1. Introduction

Natalizumab is a monoclonal antibody that significantly reduces the occurrence of relapses in relapse-remitting multiple sclerosis (RRMS) patients.(Wipfler et al., 2011) It is a recombinant antibody directed against the lymphocyte α4 subunit of the α4 β1 (VLA-4) integrin. It prevents the binding between VLA-4 and vascular endothelium cell adhesion molecule 1 (VCAM-1), which, over time, decreases α4 expression, resulting in a reduced extravasation of inflammatory immune cells across the blood-brain barrier into the central nervous system.(Miller et al., 2003).

Due to modifications of the bone marrow vascular niche and the interference of natalizumab with the homing of haematopoietic stem cells, the major haematologic finding in patients treated with natalizumab relates to the number of CD34+cells, which rapidly egress from the bone marrow cavity into the

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peripheral blood (Zohren et al., 2008).

VLA-4 also play a critical role in erythropoiesis (Hamamura et al., 1996), being essential for the terminal proliferation and differentiation of erythroid progenitor cells. Erythroid cells specifically express fibronectin receptors α4 β1, and the engagement of α4 β1 integrin by fibronectin provides signals that are necessary for the terminal expansion of differentiating erythroblasts.(Eshghi et al., 2007).

Early papers on the clinical use of natalizumab in RRMS patients reported erythroblastemia as an occasional and transient side effect (Polman et al., 2006; Ransohoff, 2007). Recently, Robier et al. reported that in 14 RRMS patients treated with natalizumab they observed a high prevalence of nucleated red blood cells (NRBCs) (93%) on blood smears, compared to 14 interferon treated patients (Robier et al., 2014).

However data are lacking on the prevalence of elevated NRBCs in wider populations of patients treated with natalizumab compared with other treatments and healthy controls. This study aimed to determine the prevalence of elevated NRBCs in peripheral blood in RRMS patients treated with natalizumab, fingolimod, interferon, glatiramer acetate, RRMS treatment naïve patients and healthy controls from the same geographic area and using the same laboratory equipment.

2. Materials and methods

2.1. Study population

We consecutively selected all RRMS patients who came to our hospital for clinical and blood analysis in our Multiple Sclerosis Center between November and December 2014. Patients with previous immunosuppressive or previous natalizumab treatment were excluded.

We collected the samples from 203 RRMS patients who were classified according to pharmacological treatment, as follows: 26 subjects currently receiving natalizumab (NTZ), 72 currently receiving interferon (IFN), 41 currently receiving glatiramer acetate (GA), 17 currently receiving fingolimod (FTY) and 47 treatment naïve patients (NA). In the NTZ group the time lapse from the last natalizumab infusion was approximately 1 month (median 31 days, IQR 28–34). Furthermore, 240 healthy subjects (HS) were randomly selected from healthy blood donors (first donation) from the same geographic area to compare their results with our RRMS patients.

2.2. Sample preparation and analysis

A total number of 443 peripheral whole blood samples (203 RRMS patients and 240 HS) collected in K_3EDTA blood tubes (Becton Dickinson, Franklin Lakes, NJ) and processed on XN-9000 (Sysmex Co., Kobe, Japan) were analyzed. The cell blood count (CBC) and extended leukocyte differential count was always performed within 2 hours from sample collection (blood draw).

XN-9000 analyzer has a specific channel (WNR) for NRBCs counting based on optical fluorescence system associated with a specific lysing agent which is responsible for selective red blood cells (RBCs) lysis. NRBCs counts are expressed both as percent (%) and absolute (#) count (x10^9/L) (Briggs et al., 2012a).

The imprecision of the NRBC automated count was assessed according to the Clinical and Laboratory Standards Institute (CLSI) EP5-A2 guideline (McEnroe et al., 2014), by analyzing three different levels (1, 2 and 3) of XN-CHECK quality control material (Streck Laboratories Inc., Omaha, NE, USA). These controls were analyzed in duplicate for 40 consecutive working days. The imprecision, expressed as coefficient of variation, was below 9.5%. The blood smears were automatically prepared with Autoslider SP-10 slide maker (Sysmex Co., Kobe, Japan) and then stained with May-Grünwald-Giemsa(Carlo Erba Reagents S.p.A. Milano, Italy).

The blood smear review process was performed with DI60 (Sysmex Co., Kobe, Japan). Both Autoslider SP-10 and DI60 were physically connected with the XN-9000 analyzer.

The digital images were then reevaluated and validated by a skilled specialist in laboratory hematology, according to the CLSI standard H20-A2 (Koepke et al., 2010) and ICSH guideline (Briggs et al., 2014). Samples were confirmed positive on microscopic review when NRBCs were $\geq$1/200 white blood cells (WBCs) (or $\geq$0.5%) in accordance with the criteria described in Standard International Consensus Group for Hematology (Barnes et al., 2005).

In the NTZ group only, we carried out an additional analysis on a subsequent blood sample in order to count the CD34+ cells as markers of bone marrow mobilization. The cell enumeration was performed using the BD™ Stem Cell Enumeration Kit (Becton Dickinson., cat 344563 CE IVD). We incubated 100 μL of peripheral blood with 20 μL of CD45 FITC/CD34 PE reagent and 20 μL of 7-AAD at room temperature. The BD FACSCanto II Flow Cytometer acquired the data, which were analyzed by the BD FACSCanto Software, meeting the ISHAGE guidelines for cell count in single platform (Sutherland et al., 1996).

2.3. Statistical analysis

For each treated patient we considered for NRBC analysis only single blood samples drawn between November and December 2014. For NA patients we analyzed the results of the first available blood sample of year 2014.

Statistical analyses were performed by SPSS statistical software v. 17.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were estimated by the non-parametric Kruskal-Wallis test (with Bonferroni correction for multiple comparisons). Differences in proportions were estimated by Fisher's exact test. Predictors and the effect of confounding factors for NRBC positivity ($>0.001\times10^9$/L) were estimated by univariate and multivariate logistic regression (full model): only predictors significantly ($p<0.05$) associated to NRBC positivity at the univariate analysis were considered in the multivariate model.

2.4. Standard protocol approvals, registration, and patient consent

The study was conducted in accordance with the Helsinki Declaration and under the terms of all relevant local legislations. The investigation was based on pre-existing samples; approval was obtained from the local ethical committee.

3. Results

The rate of samples with NRBCs (threshold of positivity: 0.001×10^9/L and confirmed by microscopic review) was significantly higher in patients receiving natalizumab (NTZ) (92%) compared with all the other treatment groups (0% for FTY, 1% for IFN, 7% for GA, 2% for NA) and HS (0%) ($p<0.0005$) (Table 1).

The median value of NRBCs, WBCs, RBCs, NRBCs, haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and red cell distribution width (RDW) of all subjects are shown in Table 1.

The median value of NRBCs count was significantly higher in NTZ (median 0.020×10^9/L, $p<0.01$) than in all other treatment groups (median 0) (Table 1).

The median values of WBCs in NTZ were significantly higher than all other groups ($p<0.01$) (Table 1).