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Highly Sensitive Chemiluminescent Method for the Detection of Maternal Cell Contamination in Human Cord Blood Stored for Allotransplantation: The Experience of the Milano Cord Blood Bank

Francesca Poli, Loretta Crespiatico, Lucilla Lecchi, Girolamo Sirchia, Mario Scalamogna, Silvia Maria Sirchia, Isabella Garagiola and Laura Pedranzini

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CORRESPONDENCE

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To the Editor:

Among the questions to be answered regarding umbilical cord blood (CB) cell use for bone marrow reconstitution, one concerns the level of contamination with maternal lymphocytes that could be responsible for severe graft-versus-host disease (GVHD) in immunosuppressed recipients.[1,2]

In the present study, we report data concerning the contamination with maternal cells in 50 consecutive CB units; to this purpose, we used polymerase chain reaction (PCR) amplification of minisatellite sequences followed by chemiluminescent detection.

CB was collected after delivery of term newborns as previously described.[3] Genomic DNA was extracted with the salting out method[4] from 500 μL of CB and mother's EDTA whole blood. Samples were handled in the preamplification area in a laminar vertical air flow hood using dedicated positive displacement pipettes.

Apolipoprotein B gene (ApoB)[5] and D1S80[6] minisatellite analysis was performed.

Firstly, we performed a PCR assay of all samples using primers that amplify the VNTR 3′ of the ApoB gene. The VNTR was considered to be not informative when the mother was homozygous or had the same alleles as the child. When ApoB was not informative, we amplified the D1S80 polymorphic locus.

Each PCR reaction was performed in 50 μL final volume containing 100 ng of genomic DNA, 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 9.0, 1.5 mmol/L $MgCl_2$, 0.1% Triton X-100, 0.15 μmol/L (for ApoB) or 0.4 μmol/L (for D1S80) of each primer, 36 μmol/L of dCTP, 100 μmol/L of each dNTP (of a mix containing dATP, dGTP, and dTTP), 60 μmol/L of biotin-labeled dCTP, and 3 U of Taq DNA polymerase. PCR conditions and primer sequences were those described elsewhere for ApoB[5] and for D1S80.[6]

Seven microliters of PCR product was loaded on a 3% agarose gel and electrophoresed for 16 hours with 1× TBE at 7 V/cm at 4°C.

To assess the sensitivity of our test and to determine the magnitude of the contamination with maternal cells in CB samples, standards obtained mixing 50 ng/μL of DNA from two individuals with alleles of different sizes for each VNTR polymorphism (ApoB, D1S80) at the dilutions 1:500, 1:1,000, and 1:2,500 were amplified in parallel with the other samples and loaded on the gel. After electrophoresis, DNA was transferred from the gel into a Tropilon plus positively charged nylon membrane (Tropix, Bedford, MA) by Southern blotting.

DNA fragments generated in the PCR reaction were labeled through the incorporation of biotinylated dCTP. The detection of PCR products was obtained through two steps: (1) the conjugation of streptavidin-alkaline phosphatase conjugate (Avidix-AP; Tropix) to the biotin-labeled PCR products; and (2) the addition of CSPD^R substrate (Tropix), which decomposed upon enzymatic dephosphorylation. The light emission, from the enzyme activated CSPD^R substrate resulted in a DNA band pattern that was imaged on x-ray film (Kodak X-Omat AR; Eastman Kodak Co, Rochester, NY).

For the detection of PCR products, the CSPD^R protocol Southern-Light Nucleic Acid Detection Systems (Tropix) was followed.[7,8]

Exposure to x-ray film ranged from 15 minutes to 2 hours, depending on the visualization of the contaminating band in the standard diluted 1:2,500. Every test was performed twice to confirm the results.

For the 50 cases examined, ApoB was informative for 21 samples, whereas for the remaining samples D1S80 was useful.

Noninherited maternal allele was detected in 5 of the 21 CB samples examined with ApoB; with D1S80, 5 additional samples were found contaminated of the 29 informative.

The level of contamination, defined comparing the intensity of the contaminating band with that of the standards, ranged from 1:100 to 1:2,500.

The child paternal allele was detected in 3 of the 30 mothers whose newborn was heterozygous at the loci examined.

Figure 1 reports an example of ApoB minisatellite analysis.

The problem of contamination of CB units with maternal lympho-

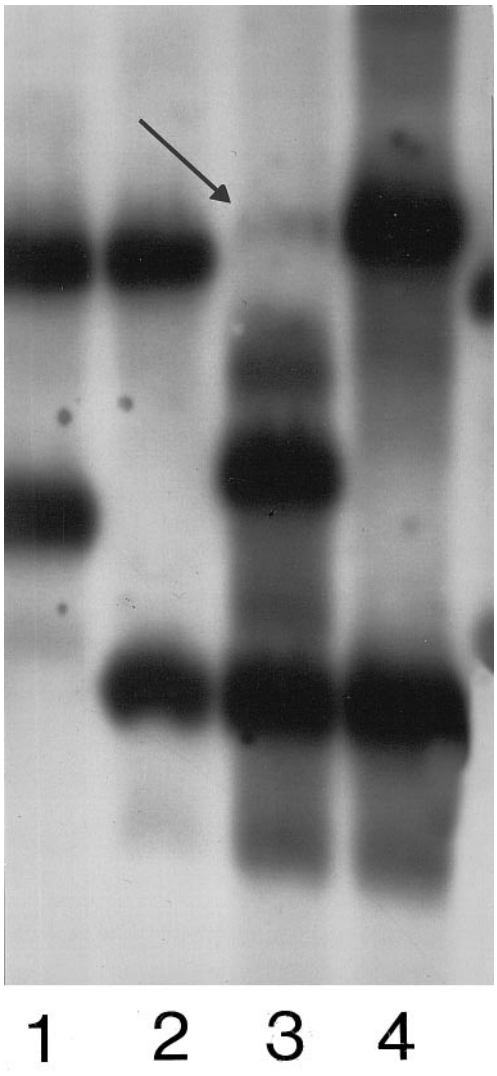

Fig 1. Lane 1 shows a noncontaminated cord examined with ApoB. Lane 2 shows the respective mother. Lane 3 reports a contaminated cord. Lane 4 shows the mother. The arrow indicates the band due to maternal DNA contamination.

cytes has been widely investigated. However, to our knowledge, the results available[9-13] refer to numerically limited series and, to a certain extent, are discordant depending on the method used.

The method we used was able to detect maternal contamination from as few as 2×10^4 nucleated cells with a sensitivity of 0.04%, comparable to that reported by Sociè et al,[12] without resorting to radioactivity.

Moreover, we were able to demonstrate an exchange of cells from child to mother through the placenta/uterus interface, supporting previously published studies that have shown the passage of fetal cells into the maternal circulation during pregnancy.[10,14]

However, we are aware that our series is limited, too; the combination of minisatellite amplification and chemiluminescence has never been used to this purpose and it proved to be highly sensitive.

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Francesca Poli
Loretta Crespiatico
Lucilla Lecchi
Girolamo Sirchia
Centro Trasfusionale e di Immunologia dei Trapianti
Mario Scalamogna
Servizio per il Prelievo e Conservazione di Organi e Tessuti
IRCCS
Ospedale Maggiore Policlinico
Milano, Italy
Silvia Maria Sirchia
Isabella Garagiola
Laura Pedranzini
Fondazione Centro Studi Patologia Molecolare Applicata alla Clinica
Milano, Italy

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Mycosis Fungoides and Total Skin Electron Beam Radiation

To the Editor:

We are writing to clarify comments concerning total skin electron beam radiation (TSE) made by Diamandidou et al[1] in their comprehensive review of mycosis fungoides and the Sezary syndrome.

The technical description of TSE in the review did not distinguish between premodern and modern methods of TSE. Modern TSE is defined by a skin surface dose of 35 to 36 Gy delivered with electrons of at least 4 MeV energy.[2-4] This TSE optimizes the distribution of dose within the main target volume (epidermis and dermis, to a depth of 5 mm; including blood present in the skin during radiation). It also increases the penetration of electrons to between 2 to 3 cm to encompass many superficial lymph nodes.[2] These attributes are clinically significant. A meta-analysis of the main technical parameters of TSE showed that modern TSE is associated with very high rates of complete remission, with even advanced disease having a rate in excess of 70%.[2,3] Of course, a key question is whether modern TSE improves survival as compared with premodern TSE or no TSE.

The survival experiences of patients receiving less optimal versions of TSE were compared with the experiences of patients managed with other topical therapies in 4 studies. These included contemporaneous,[5] concurrent,[4,6] and randomized[7] controls. All 4 studies showed that premodern TSE achieved only moderate rates of complete remission and was not associated with improved survival. For example, in the randomized trial,[7] combined premodern TSE and systemic chemotherapy was no better than conservative management in 103 randomized patients, although it should be noted that a majority of the patients in this trial had nodal and visceral disease (and were stage IV). In contrast, both Hoppe et al[8,9] and Jones and Thorson[4] have separately shown that more optimal versions of TSE are associated with improved progression-free and overall survivals.[3,8] Kim et al[10] have just reported a comparison of mechlorethamine versus a predominantly historical and essentially premodern TSE control group for only stage IA disease. None of 34 TSE patients and 3 of 73 mechlorethamine patients died of MF (5% difference at 10 years; $P = .18$). Recently, Jones and Thorson[4] were able to