

# Fluorescence in situ hybridization and chromosome studies after transfusion in newborns: Is a waiting period necessary?

Jennifer Fynn, MD<sup>1,2</sup>,\* Violeta Komotos, MD<sup>1,2</sup>,\* Debra Rita, MD<sup>1,4</sup>, Raghbir Benawra, MD<sup>1,2</sup>, Lorene Schweig, RN<sup>1,3</sup>, Denise Angst, DNSc<sup>1,3</sup>, and Bhagya Puppala, MD<sup>1,2</sup>

**Background:** Delaying chromosome studies after transfusion is common practice in many neonatal intensive care units (NICUs). Yet, no evidence exists to support this practice. **Purpose:** To investigate the effects of filtration and irradiation on chromosome detection, and to evaluate donor chromosome interference after transfusion. **Methods:** Packed red blood cells (PRBCs) were evaluated by fluorescence in situ hybridization (FISH) and chromosome analyses. To evaluate donor leukocyte survival, blood was collected from female neonates who received male-donated PRBCs. **Results:** Irradiated, leukodepleted blood had no Y chromosome detection by FISH. Irradiated, microaggregate filtered blood had Y chromosome detection in all samples by FISH but no metaphase growth. No donor chromosomes were detected in neonates after transfusion. **Conclusions:** Delaying chromosome or FISH analysis in transfused neonates who have received irradiated blood is unnecessary. *Genet Med* 2005;7(1):54–57.

**Key Words:** chromosome, in situ hybridization, fluorescence, packed red blood cells, transfusion, neonate.

\*Both authors contributed equally in this study.

Critically ill infants admitted to a neonatal intensive care unit who are anemic shortly after birth may receive blood transfusions before a chromosomal anomaly is suspected. Standard practice at many institutions is to wait at least 1 week after blood transfusion to check chromosomes in infants with suspected chromosomal anomalies. This practice delays diagnosis and increases parental anxiety. The rationale for this practice is likely to ensure the elimination of any donor cells in the recipient's circulation that could interfere with chromosomal analysis. To date, however, there have been no studies documenting that a 1-week waiting period is necessary.

In 1977, Schechter et al.<sup>1</sup> examined the clearance of white blood cells (WBCs) in adult patients after blood transfusions from opposite sex donors. In 6 of 10 adults, circulating donor lymphocytes were detected for up to 1 week after transfusion. The blood that was given to these patients, however, was not irradiated or leukocyte depleted.<sup>1</sup>

Treatment of blood products before transfusion has become increasingly common since Schechter's study and can include irradiation, microaggregate filtration, and leukocyte depletion.

Irradiation of packed red blood cells (PRBCs) serves to block the multiplication of lymphocytes, thereby decreasing the risk that the recipient develops transfusion-associated graft-versus-host disease.<sup>2</sup> Microaggregate filtration provides a filter to protect patients from microaggregates, clots, and particulate debris from stored or salvaged blood components, and leukodepletion removes leukocytes from PRBCs before transfusion to the patient.<sup>3</sup>

Filtering methods as well as practice patterns around the treatment of blood before neonatal transfusion vary among institutions. Guidelines concerning blood transfusions published by the Committee of Red Blood Cell Administration Practice Guideline Development Task Force of the College of American Pathologists (1998) recommend irradiation of blood products for low-birth weight infants (<1200 g) and for infants who are undergoing exchange transfusions or extracorporeal membrane oxygenation. However, leukocyte depletion is only recommended in neonates who are immunocompromised, have various congenital anemias or known HLA alloimmunization, or have experienced recurrent severe febrile hemolytic transfusion reactions. Also, leukocyte-reduced red blood cell units are recommended as an alternative to cytomegalovirus-seronegative components.<sup>4</sup> However, the use of irradiated and leukocyte-depleted blood products is becoming more common practice in neonates in an effort to minimize complications associated with blood transfusions. In transfusion guidelines published from the United Kingdom in 2004, all blood components in the United Kingdom are leukodepleted since November 1, 1999 in an attempt to decrease the

From <sup>1</sup>Advocate Lutheran General Children's Hospital, Department of Pediatrics, Divisions of <sup>2</sup>Neonatology, <sup>3</sup>Research, and <sup>4</sup>Genetics, Park Ridge, Illinois. Present address for J.F. is Healthfirst Family Care Center, Fall River, Massachusetts.

Violeta Komotos, MD, Lutheran General Children's Hospital, 1775 Dempster Street, Park Ridge, IL 60068.

Received: August 3, 2004.

Accepted: October 1, 2004.

DOI: 10.1097/01.GIM.0000151151.04087.57

risk of transfusion-transmitted variant Creutzfeldt-Jacob disease.<sup>5</sup>

More recently, Wang-Rodriguez and colleagues conducted a study in neonates using irradiated, leukocyte-depleted blood and found that 4 of 6 infants effectively cleared donor WBCs within 24 hours after transfusion.<sup>6</sup> This study did not address how leukocyte survival could influence chromosome studies in transfused neonates.

At present, it is unclear what quantity of donor leukocytes survive after PRBC transfusion and whether any waiting period before performing chromosome studies on the transfused neonate is warranted. The purpose of the present study was to investigate the detection of donor chromosomes in irradiated blood that was either leukodepleted or microaggregate filtered and determine the length of time that donor leukocytes could potentially interfere with chromosome or FISH analyses on the recipient's blood. Our hypothesis was that donor leukocyte chromosomes would not interfere with chromosome or FISH analyses on cultured cells, and therefore, diagnostic testing could be performed any time after transfusion.

## METHODS

This prospective, exploratory study was conducted in a Level III NICU within a Children's Hospital in the Midwest. The study protocol and consent forms were reviewed and approved by the Institutional Review Board, and for the *in vivo* study, parental consent was obtained before enrolling infants. All blood samples were filtered using microaggregate (Ultipor Blood transfusion Filter SQ40S, Pall Biomedical) or leukocyte reduction (Purecell Neonatal High Efficiency Leukocyte Reduction Filter, Pall Biomedical) filters. For the *in vivo* study, female infants who received male-donated, microaggregate filtered blood were included due to the ease of Y chromosome detection. Female infants who received multiple transfusions were excluded from the study.

### FISH studies on PRBCs

Samples were collected from leftover male-donated, irradiated PRBCs that were filtered using leukocyte depletion ( $n = 19$ ) or microaggregate ( $n = 12$ ) filters. All samples were cultured and harvested per standard practice in the genetics laboratory. A hemogram was run on all samples in order to obtain a WBC count.

For blood that was leukodepleted, slides were prepared for FISH analysis using a Vysis Chromosome Enumeration DNA FISH probe (CEP) for the Y chromosome along with a normal male control slide. The CEP Y ( $\alpha$  satellite) DNA probe hybridizes to the centromere of human chromosome Y (band region Yp11.1-q11.1, locus DYZ3). The slides were viewed under a fluorescence microscope by two examiners (one blinded and one unblinded). For blood that was microaggregate filtered, slides were prepared for FISH analysis using a Vysis Chromosome Enumeration DNA FISH probe (CEP) for both the X and Y chromosomes. These slides were viewed under the fluorescence microscope by two examiners (one blinded and one un-

blinded). The X chromosome FISH served as the internal control.

### Chromosome studies on PRBCs

Samples were collected from male-donated, irradiated, microaggregate-filtered PRBCs before transfusion into neonates ( $n = 7$ ). These samples were cultured and harvested for metaphase chromosome analysis as per standard practice in the genetics laboratory. Slides were G-banded and stained (Giemsa and Wright) for chromosome studies.

### FISH studies on transfused neonates

Blood samples were collected from 9 female neonates who received 10 cc/kg of male-donated, irradiated, microaggregate-filtered PRBCs at 12 hours, 24 hours, 48 hours, 72 hours ( $\pm$  2 hours), and/or 1 week after transfusion. The number of samples per subject varied based on the timing of the transfusion and parental consent. Subjects were excluded if additional blood transfusions were required during the study period (Table 4). All slides were examined for interphase cells and 100 nuclei per slide were counted. The Vysis Chromosome Enumeration DNA FISH probes (CEP) for the X and Y chromosomes were used. The Y chromosome FISH was chosen because it would provide clear evidence of circulating male donor leukocytes in the female recipient, whereas the X chromosome FISH probe provided an internal control.

## RESULTS

### Irradiated, leukodepleted PRBCs

WBC counts on the 19 leukodepleted samples ranged from 0.003 to  $0.835 \times 10^3/\text{mm}^3$ . FISH did not detect any nuclei with a Y signal in any of these samples (Table 1).

### Irradiated, microaggregate-filtered PRBCs

WBC counts on the 12 samples that were irradiated and microaggregate-filtered ranged from  $2.14 \times 10^3/\text{mm}^3$  to  $6.64 \times 10^3/\text{mm}^3$  (Table 2). FISH analysis of interphase cells revealed significant fluorescence detection of the Y chromosome using the CEP Y CEP X probe, with 100 nuclei counted per slide. Chromosome studies yielded no metaphase growth in any of the samples ( $n = 7$ ), preventing further chromosome analysis.

### In vivo detection

There was no fluorescence detection of the Y chromosome on any of the slides for each experiment made from the blood collected at 12, 24, 48, 72, and 168 hours after transfusion. Demographics of the nine neonates involved in the *in vivo* study are shown in Table 3. They had a mean birth weight of  $892.8 \pm 176.5$  g, a mean gestational age of  $26.9 \pm 1.2$  weeks, and a median age at transfusion of 13 days.

**Table 1**

Irradiated, leukodepleted blood samples: WBC and FISH results

Sample number	WBC $\times 10^3/\text{mm}^3$	Number of nuclei positive-Y probe signal
1	0.006	No scorable nuclei
2	0.835	No scorable nuclei
3	0.045	No scorable nuclei
4	0.007	No scorable nuclei
5	0.150	No scorable nuclei
6	0.012	No scorable nuclei
7	0.009	No scorable nuclei
8	0.009	No scorable nuclei
9	0.035	No scorable nuclei
10	0.003	No scorable nuclei
11	0.011	No scorable nuclei
12	0.017	No scorable nuclei
13	0.054	No scorable nuclei
14	unavailable	No scorable nuclei
15	unavailable	No scorable nuclei
16	0.007	No scorable nuclei
17	0.034	No scorable nuclei
18	0.003	No scorable nuclei
19	0.007	No scorable nuclei

**Table 2**

Irradiated, microaggregate filtered blood samples: WBC and FISH results

Sample number	WBC $\times 10^3/\text{mm}^3$	Number of nuclei positive-Y probe signal
1	2.69	>100
2	4.92	>100
3	4.40	>100
4	4.07	>100
5	2.14	>100
6	6.42	>100
7	5.15	>100
8	4.96	>100
9	5.49	>100
10	6.64	>100
11	5.81	>100
12	6.43	>100

## DISCUSSION

Neonates requiring intensive care are among the most frequently transfused group of patients. It has been estimated that about 38,000 low birth weight infants are born in the United States annually and approximately 80% of these infants will

**Table 3**

Female infant demographics: In vivo study after transfusion

Subject	Gestational age at birth (weeks)	Birth weight (grams)	Age at transfusion
1	25 $\frac{2}{7}$	753	1 day
2	25 $\frac{1}{7}$	660	10 days
3	27 $\frac{3}{7}$	932	13 days
4	27 $\frac{3}{7}$	754	12 days
5	28 $\frac{5}{7}$	1075	14 days
6	27 $\frac{5}{7}$	1155	12 days
7	27 $\frac{3}{7}$	927	45 days
8	27 $\frac{3}{7}$	1050	51 days
9	26 $\frac{0}{7}$	729	24 days

receive multiple PRBC transfusions before discharge.<sup>7</sup> Occasionally, neonates may be suspected of having chromosomal anomalies requiring chromosome or FISH analysis after they have already received an emergent PRBC transfusion.

Currently, there are no specific guidelines regarding the timing of chromosome analysis after transfusion. Moreover, filtering methods and treatment of blood before neonatal transfusion still vary greatly between institutions. An informal survey of NICUs participating in the Vermont Oxford Network conducted by one of the authors revealed different filtering practices before transfusion and varied timing of chromosome studies after transfusion, ranging from no waiting period to a waiting period of several months.

In a retrospective study, Kulharya et al.<sup>8</sup> reviewed the medical records of 10 newborn infants who had received blood transfusions between 1 to 10 days before routine cytogenetic analysis. Three infants received irradiated and leukodepleted blood, four infants received irradiated blood, and three infants received blood that was neither irradiated nor leukodepleted. Because there were no instances where cells with a karyotype of the opposite sex were detected in the neonates' blood samples, the authors concluded PRBC transfusions did not compromise the accuracy of chromosome analysis.<sup>8</sup> Because this study was retrospective, there was no documentation on which patients received blood from opposite sex donors. The authors concluded that the likelihood of study patients receiving blood from a donor of the opposite sex was 50% based on the 1:1 male to female donor ratio in the blood bank. This study was limited by the uncertainty of the blood donors' sex and the small sample size; however, the results of this study are consistent with our current study.

Other research has examined donor WBC clearance in neonates after blood transfusions. In a study by Wang-Rodriguez et al.,<sup>6</sup> they found no evidence of male donor cells in two female neonatal subjects one day after receiving irradiated, leukodepleted, male-donated blood when measured by semiquantitative polymerase chain reaction (PCR) Y-chromosome technique. They also found that two out of four female infants that

**Table 4**

Y chromosome signal detection in female neonates after transfusion of male-donated, irradiated, microaggregate filtered blood

Subject	Y chromosome signal detection				
	12 hours	24 hours	48 hours	72 hours	1 week
1	2X,0Y	2X,0Y	2X,0Y	2X,0Y	2X,0Y
2	*	*	2X,0Y	2X,0Y	2X,0Y
3	2X,0Y	2X,0Y	2X,0Y	2X,0Y	*
4	*	2X,0Y	2X,0Y	2X,0Y	*
5	2X,0Y	2X,0Y	2X,0Y	2X,0Y	2X,0Y
6	2X,0Y	2X,0Y	2X,0Y	2X,0Y	2X,0Y
7	*	2X,0Y	*	*	*
8	*	2X,0Y	*	*	*
9	*	2X,0Y	*	*	*

\*Specimen unavailable for analysis.

received irradiated but nonleukodepleted blood had detectable male donor cells on the first day after transfusion, but subsequent samples taken after 24 hours had no detectable donor WBCs.<sup>6</sup> These findings suggest that neonates have the capability to rapidly clear donor WBCs that have been irradiated and that leukodepletion seems to effectively reduce donor WBC detection in the transfused patient.

Clearly, the treatment of blood before transfusion affects the detection and length of survival of donor cells in the recipient. Early methods for investigation of donor leukocyte survival involved karyotype analysis of posttransfusion samples in opposite sex donors and recipients where blood was neither irradiated nor leukodepleted. Hutchinson and colleagues found that lymphocytes from random donors were detectable up to several weeks after exchange transfusion, whereas lymphocytes from a mother's blood were detected for as long as 2 years in their recipient sons secondary to in utero fetomaternal hemorrhages.<sup>9</sup>

More recently, Lee et al.<sup>10</sup> demonstrated that the length of donor WBC survival in recipients is influenced by the treatment of blood products before transfusion. They studied 4 adult females who received leukodepleted but not irradiated blood. Using SRY-specific PCR amplification, they found that all subjects had a transient detection of male-donated WBCs during the first week after blood transfusion and complete clearance of donor WBCs by 2 weeks after transfusion.<sup>10</sup>

These results, along with our current work, suggest that when PRBCs are irradiated and filtered (using either leukocyte depletion or microaggregate methods), delaying chromosome studies after transfusion is unnecessary. Our study revealed that donor cells will not interfere with chromosome analysis or FISH analysis on cultured cells 12 hours after transfusion. Although we do not suspect that our results would have been different if we had studied the recipient's blood within 12 hours of transfusion, this was not evaluated in the current

study. Also, we did not address the issue of using direct FISH in transfused neonates for rapid detection of common trisomies. In these cases, interference of donor cells may present the clinician with low-order mosaicism for which clinical judgment and interpretation is needed.

#### ACKNOWLEDGMENTS

This research was supported by a grant from the Park Ridge Community Fund. We thank Henry H. Mangurten, MD for his critical review of our manuscript. We would also like to thank the members of the Genetics Lab at Advocate Lutheran General Hospital for their support as well as the NICU nursing and medical staff for their assistance in obtaining the blood samples. We would also like to thank all of the families of the infants that consented to participation in this study.

#### References

- Schechter G, Whang-Peng J, McFarland W. Circulation of donor lymphocytes after blood transfusion in man. *Blood* 1977;49:651–656.
- Desmet L, Lacroix J. Transfusion in pediatrics. *Crit Care Clin* April 2004;20:299–311.
- Sweeney J. Universal leukoreduction of cellular blood components in 2001? *Am J Clin Pathol* 2001;115:666–673.
- Simon TL, Alverson DC, AuBuchon J, Cooper ES, DeChristopher P, Glenn G, et al. Practice parameter for the use of red blood cell transfusions. *Arch Pathol Lab Med* 1998;122:130–138.
- Gibson B, Todd A, Roberts I, Pamphilon D. Transfusion guidelines for neonates and older children. *Br J Haematol* 2004;124:433–453.
- Wang-Rodriguez J, Fry E, Fiebig E, Lee T, Busch M, Mannino F, et al. Immune response to blood transfusion in very-low-birthweight infants. *Transfusion* 2000;40:25–34.
- Ringer SA, Richardson DK, Sacher RA, Keszler M, Churchill WH. Variations in transfusion practice in neonatal intensive care. *Pediatrics* 1998;101:194–200.
- Kulharya A, Salbert B, Norris K, Cook L, Larrison P, Flannery D. Packed red cell transfusion does not compromise chromosome analysis in newborns. *Genet Med* 2001;3:314–317.
- Hutchinson D, Turner J, Schlesinger E. Persistence of donor cells in neonates after fetal and exchange transfusion. *Am J Obstet Gynecol* 1971;109:281–284.
- Lee T, Sakahara N, Fiebig E, Hirschhorn D, Johnson D, Busch M. Quantitation of white cell subpopulations by polymerase chain reaction using frozen whole-blood samples. *Transfusion* 1998;38:262–270.