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# The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes

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**The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes.**

*Background.* The incidence of pure red cell aplasia (PRCA) in chronic kidney disease patients treated with epoetins increased substantially in 1998, was shown to be antibody mediated, and was associated predominantly with subcutaneous administration of Eprex®. A technical investigation identified organic compounds leached from uncoated rubber stoppers in prefilled syringes containing polysorbate 80 as the most probable cause of the increased immunogenicity.

*Methods.* This study investigated whether the incidence of PRCA was higher for exposure to the product form containing leachates than for leachate-free product forms. Antibody-mediated PRCA cases were classified according to indication, product form, and route of administration. Exposure estimates were obtained by country, indication, route of administration, and product form.

*Results.* For 2001 to 2003, the PRCA incidence rate for patients with subcutaneous exposure to Eprex in prefilled syringes with polysorbate 80 and uncoated rubber stoppers (leachates present) was 4.61/10,000 patient years (95% CI 3.88–5.43) versus 0.26/10,000 patient years (95% CI 0.007–1.44) for syringes with coated stoppers (leachates absent). The rate difference was 4.35/10,000 patient years (95% CI 3.44–5.26; $P < 0.0001$); the rate ratio was 17 (95% CI 3.14–707). A substantial rate difference remained in sensitivity analyses that adjusted for exposure to multiple product forms.

*Conclusion.* The epidemiologic data, together with the chemical and immunologic data, support the hypothesis that leachates from uncoated rubber syringe stoppers caused the increased incidence of PRCA associated with Eprex. Currently, all Eprex prefilled syringes contain fluoro-resin coated stoppers, which has contributed to decreased incidence of PRCA with continued surveillance.

**Key words:** pure red cell aplasia, erythropoietin, antibodies, epoetin alfa.

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Pure red cell aplasia (PRCA) is a rare disorder that manifests itself as a severe, isolated anemia of sudden onset, characterized by an almost complete absence of red cell precursors in the bone marrow and a reticulocyte count below $10 \times 10^9$/L [1]. Many potential causes for PRCA have been reported, but most concern only isolated case reports, and about 50% of cases have no known cause [2]. Over the decade following its introduction in 1989, three cases of PRCA were associated with recombinant human erythropoietin (epoetin) treatment for anemia in patients with chronic kidney disease (CKD) [3–5]. From 1998 onward, however, an increasing number of cases were reported [2]. These patients developed PRCA due to neutralizing antibodies to epoetin that cross-react with endogenous erythropoietin, and therefore, the condition is termed antibody-mediated PRCA.

These cases occurred mainly in patients receiving epoetin alfa (EPREX®/ERYPO®; Ortho Biotech, a division of Janssen-Cilag, Bridgewater, NJ, USA) outside the USA, although a limited number received other epoetin products or a combination of products [6–10]. Irrespective of the type of epoetin administered, virtually all of the cases occurred in CKD patients treated with subcutaneous epoetin; no cases have occurred in cancer patients [10]. To limit the increasing incidence of antibody-mediated PRCA attributed to Eprex, risk-mitigation initiatives were taken, including improved cold chain storage and handling of Eprex [11], and a switch to intravenous administration for CKD patients. Health authorities in Europe formally contraindicated subcutaneous administration of Eprex for CKD patients in December 2002.

Technical and clinical investigations were initiated to identify the cause of the observed increase in the immunogenicity of Eprex. Investigations into the manufacturing process for bulk drug substance and characterization of Eprex packaged into prefilled syringes did not uncover any irregularities in the manufacturing process

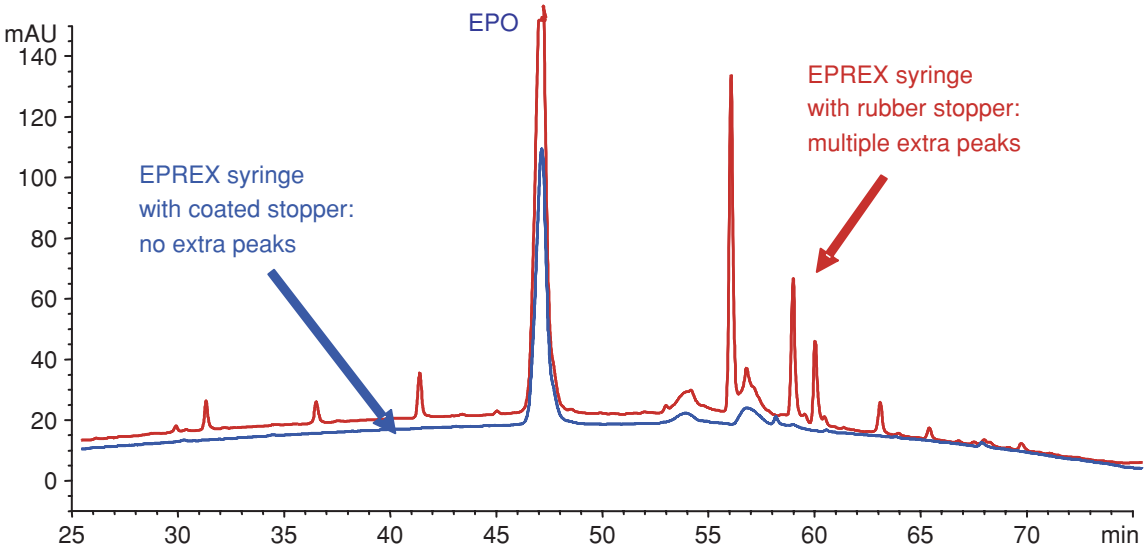


**Fig. 1. Reverse-phase high-performance liquid chromatography.** The large peak at 47 minutes is epoetin (EPO). The small peaks at 54 and 57 minutes are polysorbate 80. A series of extra peaks are present in the polysorbate 80 formulation of Eprex dispensed from prefilled syringes with uncoated rubber stoppers (red) that are not present in the same product dispensed from syringes with coated stoppers (blue). Reproduced with modification from Sharma et al with permission from the European Journal of Hospital Pharmacy [12].

or finished product that could be linked to the increased incidence of PRCA [12]. During the course of this systematic investigation, however, a series of peaks were observed on an experimental high-performance liquid chromatography (HPLC) elution profile of Eprex from prefilled syringes (Fig. 1) [12]. These peaks occurred after the large epoetin peak and were identified by mass spectrometry as organic compounds present in the rubber stoppers [12]. It was reported in 2002 that polysorbates and other nonionic detergents can leach compounds out of plastics and rubber materials [13]. These leachates were only detected in the polysorbate 80 formulation of Eprex from prefilled syringes with uncoated rubber stoppers. Eprex preparations containing polysorbate 80 with fluoro-resin coated stoppers (FluroTec®, Daikyo Seiko, Tokyo, Japan) did not demonstrate these leachates, nor did those with uncoated stoppers containing human serum albumin (HSA) instead of polysorbate 80 as stabilizer. Leachates were not present in other epoetin products, all of which have coated stoppers. These data suggest that the polysorbate 80 in the formulation had extracted these compounds from the uncoated rubber stoppers [12]. The concentration of leachates in the syringes was variable and increased with time and exposure to heat [12]. Studies using the well-characterized antigen ovalbumin demonstrated that these leached compounds could act as adjuvants when administered subcutaneously in mice [12].

The in vivo findings for the adjuvant potential of leachates led to the hypothesis that leachates present in pers increased the risk of PRCA when this product was administered subcutaneously in humans. To determine if this hypothesis was consistent with the clinical data, the incidence of PRCA by formulation, product form, and route of administration were determined in a retrospective study using reported PRCA cases. This was possible because prefilled syringes with coated stoppers had already been introduced in 2001 for some dosage strengths and, in 2003, all dosage strengths of prefilled syringes with the polysorbate 80 formulated Eprex were shipped with coated stoppers.

## METHODS

### Reverse-phase HPLC

Reverse-phase HPLC analysis was carried out as previously described [12]. Briefly, the contents of epoetin alfa syringes were injected into a Vydac C4 column. After a 5-minute hold at 5% mobile phase B (0.06% trifluoroacetic acid in acetonitrile), samples were eluted at a flow rate of 1.0 mL/minute by a linear gradient of 5% to 90% of mobile phase B for 90 minutes. Monitoring was performed at 280 nm.

### Pharmacovigilance

An expanded review was undertaken of all spontaneous adverse events reported to Johnson & Johnson relating to any epoetin product and any indication for use, gathered from physicians, pharmacists, the literature, medical representatives, and the patients

Activities' code terms was used to ensure that all possible cases of antibody-mediated PRCA were captured. Loss of effect was defined as an absent or decreased response to erythropoietin treatment in a patient who previously responded to such therapy, and characterized by an increase in erythropoietin dosage and/or a sustained decrease in hemoglobin. Antibody-mediated PRCA was defined as either suspected or confirmed PRCA with a positive anti-erythropoietin antibody test with any testing method. Suspected PRCA was defined as loss of efficacy with a decrease in hemoglobin of ≥2 g/dL in 30 days, need for transfusions, reticulocytes <20,000 per $mm^3$, platelet count and white blood cell count normal, and bone marrow unavailable or unknown. Confirmed PRCA was defined as suspected PRCA plus bone marrow with isolated erythroblastopenia.

Exposure was estimated for patients receiving Eprex (exclusively or in combination with another epoetin product) for anemia in five disease areas: chronic kidney disease receiving dialysis, chronic kidney disease not receiving dialysis, oncology, infectious disease (infection with human immunodeficiency virus), and remaining use (typically surgery). Estimates of the actual patient years of exposure were obtained for each country by year (and monthly from July 2002) for each indication, route of administration, and formulation using drug-monitoring data. The PRCA incidence rate (per 10,000 patient years) was calculated by dividing the number of cases by the corresponding exposure (in 10,000 patient years). Reports received by June 30, 2004 were included. When adjusting for market exposure for the cumulative time period, the incidence by date of onset is calculated with exposure through the end of April 2004, not to the end of June 2004. This accommodates an assumed minimum interval of two month's delay from the occurrence of LOE to the determination of PRCA diagnosis and report to the manufacturer; however, the lag time, in practice, may be longer.

### Case series analysis

Antibody-mediated PRCA cases in patients with CKD were identified in the Johnson & Johnson clinical database. The cases were attributed to Eprex if exposure was more than one month with an adequate response and, if there was a recent switch from another epoetin, there was an ongoing response to that epoetin at the time of the switch. Using information from treating physicians, Counsel for International Organizations of Medical Sciences forms, site assessments, and the company clinical database, each case was evaluated and assigned to a category based on exposure to a certain product presentation (vials or prefilled syringes containing HSA or polysorbate 80 as stabilizer, coated or uncoated rubber syringe stoppers, and subcutaneous) of Eprex. The evaluation also took into account the first date of availability for the different formulations and product forms per country.

The following criteria were predetermined for the purpose of retrospectively attributing the occurrence of PRCA to a particular product exposure or route of administration. The time frame of suspected exposure was defined as one to 12 months prior to the onset of loss of effect of Eprex. As there have been no cases of PRCA with only intravenous exposure to Eprex, the nine cases with mixed intravenous and subcutaneous exposure prior to loss of effect were classified together with cases with only subcutaneous exposure. Two cases of patients not on dialysis or on peritoneal dialysis whose route of administration was unknown were attributed to subcutaneous exposure, a reasonable assumption, as most of these patients do not routinely have intravenous access. Based on the information of product availability over time in the different countries, a decision tree was designed to allow classification of each case into a specific category. If both the product form and the dose were unknown, the case was classified as unknown.

### Statistical methods

Rate ratios, rate differences, and *P* value calculations for the case series analyses were performed with STATA version 8.0 (Stata Corporation, College Station, TX, USA). Confidence intervals for the incidence rates were based on the Poisson distribution for rare events.

## RESULTS

### Incidence

Based on reports received by Johnson & Johnson from January 1, 1989 to June 30, 2004, 217 cases of antibody-mediated PRCA were observed among patients with chronic kidney disease. Of these, 206 cases were attributed to epoetin alfa marketed under the EPREX/ERYPO trade name, 23 of which had exposure to both Eprex and another epoetin. Of the 206 cases, 192 had only subcutaneous exposure, nine had both intravenous and subcutaneous exposure, and for five, the route was unknown. Assigning those with mixed route exposure to the subcutaneous route and excluding those cases with unknown route, the overall rate of antibody-mediated PRCA was 1.61 (95% CI 1.40–1.85) per 10,000 patient years of subcutaneous exposure (201 cases/1,244,970 patient years) versus 0 (95% CI 0–0.04) per 10,000 patient years of intravenous exposure (0 cases/871,098 patient years) for the cumulative time period.

The occurrence of PRCA began to increase in 1998 and peaked from 2001 to 2002 (analyzed by date of onset rather than date of report). Subcutaneous exposure

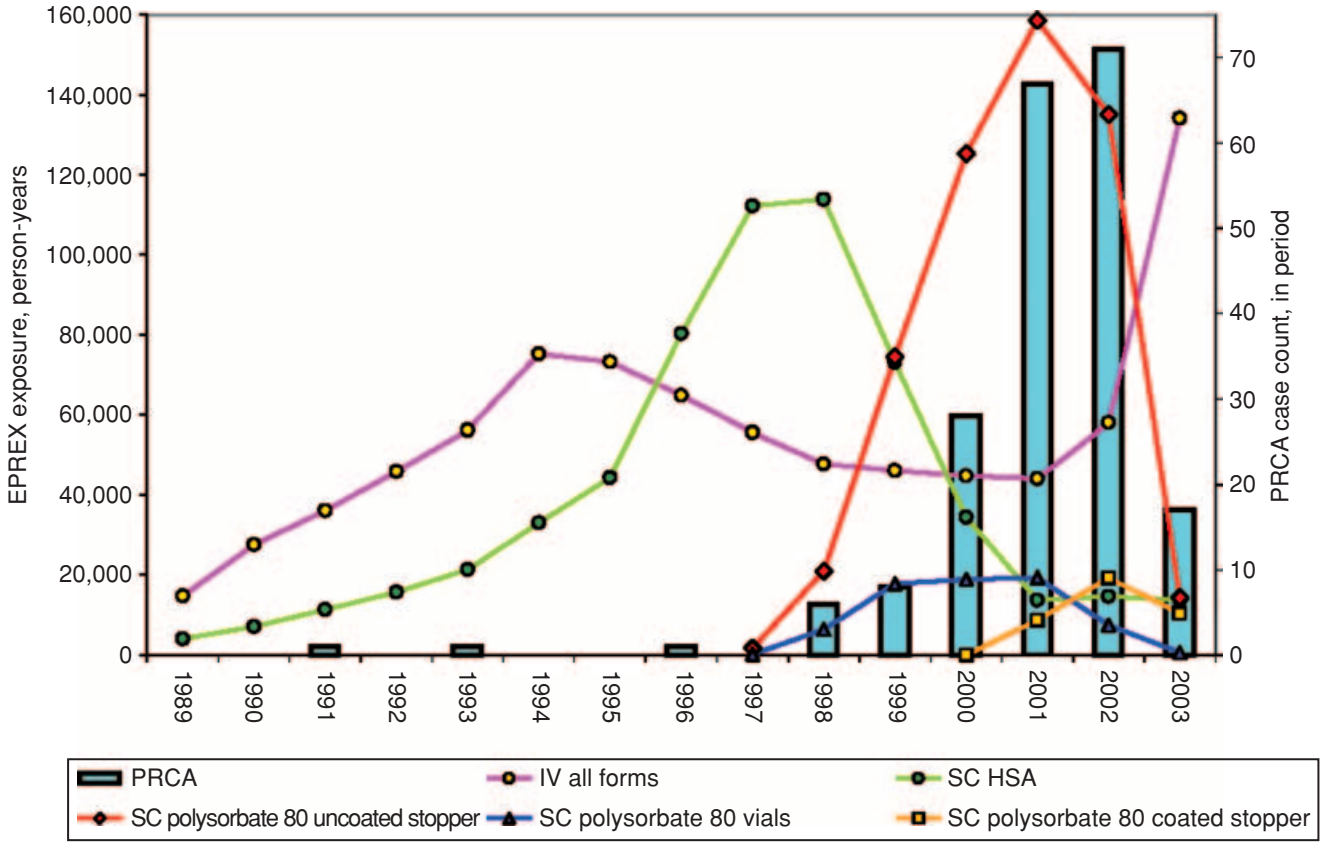


**Fig. 2. Onset of antibody-mediated pure red cell aplasia (PRCA) and worldwide Eprex exposure for nephrology by calendar time.** The bar graph shows the number of antibody-mediated PRCA cases by year in which the loss of effect occurred. Four cases are not shown due to unknown year of loss of effect. Two additional cases occurred in the period January-April 2004. Eprex exposure is shown linearly for intravenous exposure to all product forms and subcutaneous exposure to the human serum albumin formulation, the polysorbate 80 formulation in syringes with uncoated stoppers, polysorbate 80 in vials, and polysorbate 80 in syringes with coated stoppers. The polysorbate 80 formulation of Eprex was introduced in 1998.

stoppers peaked with 158,650 patient years in 2002, while onset of PRCA peaked in 2003 with 71 cases (see Fig. 2). In the period January through April 2004, two new cases were reported.

## Case series analysis

Classification of PRCA cases by formulation of Eprex within 1 to 12 months of onset is shown in Table 1. In all, 182 cases had subcutaneous exposure to prefilled syringes containing a formulation with polysorbate 80 stabilizer and uncoated rubber stoppers. In 46 of the 182 cases, an assumption was made that the product form was a prefilled syringe and not a single-use vial based on product availability in the country. Ten cases had exposure to only HSA-containing product (vials or prefilled syringes), and one had exposure to only prefilled syringes with polysorbate 80 and coated stoppers. Insufficient information was available to classify 12 cases.

Exposure to Eprex over the cumulative period 1989 exposure estimation and case ascertainment were most complete (due to publicity), and when both uncoated and coated stopper formulations were on the market, is shown in Tables 2 and 3. For the cumulative period, the incidence rate of PRCA in patients who received Eprex from syringes with polysorbate 80 and uncoated rubber stoppers was 3.43/10,000 patient years (95% CI 2.95–3.96) versus 0.23/10,000 patient years (95% CI 0.006–1.28) for products with polysorbate 80 and coated stoppers. The rate difference between these products was 3.20/10,000 patient years (95% CI 2.53–3.87; $P < 0.0001$), and the rate ratio was 15 (95% CI 2.7–594). For the period 2001 to 2003, the incidence rate of PRCA in patients who received Eprex from syringes with uncoated rubber stoppers and polysorbate 80 was 4.61/10,000 patient years (95% CI 3.88–5.43) versus 0.26/10,000 patient years (95% CI 0.007–1.44) for products with coated stoppers and polysorbate 80. The rate difference between these products was 4.35/10,000 patient years (95% CI 3.44–5.26; $P < 0.0001$), and the rate ratio was 17 (95% CI 3.14–707). Sim-

**Table 1.** Cases of antibody-mediated pure red cell aplasia in chronic kidney disease patients reported between January 1, 1989 and June 30, 2004 were classified by form of Eprex exposure

| Eprex form by subcutaneous route within 1 to 12 months of onset of loss of effect | 1989–April 2004 (total) | 2001–2003 (peak) |
|---|---|---|
| Uncoated rubber stopper, polysorbate 80 formulation, prefilled syringe | 151 | 116 |
| Coated stopper, polysorbate 80 formulation, prefilled syringe | 1 | 1 |
| Human serum albumin formulation | 10 | 2 |
| Polysorbate 80 formulation, vials | 1 | 1 |
| Mixed: Uncoated rubber stopper, polysorbate 80 formulation, prefilled syringe, and coated stopper, polysorbate 80 formulation, prefilled syringe | 27 | 25 |
| Mixed: Uncoated rubber stopper, polysorbate 80 formulation, prefilled syringe, and human serum albumin formulation | 4 | 1 |
| Unknown | 12 | 9 |
| Total | 206 | 155 |

PRCA in patients who received Eprex from syringes with uncoated rubber stoppers and polysorbate 80 against that for the subtotal of all other subcutaneous forms combined (Tables 2 and 3).

To avoid bias, the analysis was adjusted to account for mixed exposure in both the cases and the reference population. Cases with mixed exposure accounted for less than 20% of the total with known exposure. As shown in the footnotes to Tables 2 and 3, estimates for rate differences and ratios were decreased by less than 20% when cases with mixed exposure were excluded. For 2001 to 2003, approximately 25% of subcutaneous exposure to prefilled syringes with polysorbate 80 and coated stoppers was attributed to replacement of syringes with uncoated stoppers; lower mixed usage was anticipated for other forms. In sensitivity analyses, exposure estimates for product forms other than prefilled syringes with uncoated rubber stoppers and polysorbate 80 were decreased by 25% based on observed temporal trends in usage patterns. The sum of exposure subtracted from other forms was added to exposure to prefilled syringes with uncoated rubber stoppers and polysorbate 80. The rate difference between uncoated syringe stoppers and coated syringe stoppers for 2001 to 2003 was 3.89/10,000 patient-years (95% CI 2.92–4.86; $P = 0.0001$), and the rate ratio was 12 (95% CI 2.2–448) (see Table 4). Results were similar for the cumulative period 1989 to April 2004.

## DISCUSSION

Before 1998, Eprex was formulated with HSA as stabilizer. It was replaced with polysorbate 80 for most countries transmission of infectious diseases by HSA [10]. It should be noted that multidose vials containing HSA continue to be available in Canada, and prefilled syringes containing HSA are available in Turkey. Overall, the case series shows a clear temporal association between the increased incidence of antibody-mediated PRCA and the replacement of HSA with polysorbate 80 in countries outside the USA (Fig. 2). Detailed analysis of the case series revealed a substantial difference in incidence rates between patients with subcutaneous exposure to prefilled syringes with polysorbate 80 and uncoated rubber stoppers and all other product forms, suggesting that the introduction of the stabilizer polysorbate 80 in combination with the uncoated rubber stoppers had a major impact on the immunogenicity of Eprex given subcutaneously.

Subcutaneous administration of Eprex in CKD patients has decreased markedly since its contraindication in Europe; however, a low level of subcutaneous use continues. For January through April 2004, subcutaneous exposure to forms containing polysorbate 80 was 5000 patient years, and for forms containing human serum albumin, 4600 patient years. This population was included in the case series reported here. The analysis shows that the immunogenicity of Eprex in prefilled syringes with coated stoppers is substantially lower than that of the product with uncoated rubber stoppers. These findings correlate with the results of the technical investigation, which demonstrated that the combination of polysorbate 80 and uncoated rubber syringe stoppers introduced leachates from the stoppers into the product [12].

Although rubber stoppers were in use from the first introduction of Eprex prefilled syringes for subcutaneous administration in 1994, the replacement of HSA with polysorbate 80 in 1998 appears to have effected a change in the leaching of potentially immunogenic compounds from the rubber stoppers of epoetin presentations most commonly used in patients with CKD. Epoetin beta and darbepoetin syringes for subcutaneous injection were introduced after those of Eprex and have coated stoppers; no leachates were detected in these products, despite the presence of polysorbate stabilizers [12]. The enhanced immune response in mice in the presence of rubber leachates supports the hypothesis that these compounds could act as adjuvants to increase the immunogenicity of Eprex in humans [12].

The question remains why relatively few cases of antibody-mediated PRCA have occurred, and why some (a very few) cases have occurred in patients treated only with products other than Eprex. Multiple factors are required to trigger a T-cell–mediated immune response and loss of tolerance. These include the presence of a sufficient number of erythropoietin-recognizing T cells and B cells in the patient, as well as erythropoietin and an adjuvant. The relatively low frequency of cases is most likely

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