In this case report, we provide evidence for the possibility of red blood cell alloimmunization after bone-allograft transplantation (1). A 13-year-old boy with spastic diplegia probably caused by hypoxic encephalopathy at birth, had to undergo two surgeries because of impending bilateral hip-luxation. The surgeries were scheduled 5 months apart; during both he received femoral bone allografts. He had no history of blood transfusion, also during these surgeries no blood products were administered. Prior to implantation, the bone-allografts, recovered from living donors and stored at -80 °C for at least 6 months, were washed in saline and antibiotics. The allograft used during the first surgery was approximately 75 g in weight and 7 mL in volume.

The patient had bloodgroup 0, with rhesus phenotype ccdee and K negative; initial screening was negative. However, prior to the second surgery three RBC antibodies were identified: anti-D (2+ in LISS, 4+ in papainized cells), anti-E (1+ in LISS, 3/4+ in papainized cells) and anti-C (2/3+ in LISS, 4+ in papainized cells). DAT was negative. The only reasonable explanation for immunization was the first bone allograft. This was confirmed by the blood group and rhesus phenotype of the first donor: bloodgroup 0, rhesus phenotype CcDee and K negative. Screening was repeated 6 weeks after the second hip surgery in order to confirm the first positive screening and was again positive for anti-D, anti-C and anti-E (similar reaction strengths).

Discussion
With this case report, we show that also transplantation of nonvascularized tissue, such as a femoral bone allograft, may cause RBC alloimmunization. It is unlikely that intact RBCs or bone marrow stem cells are still present in the used allograft. However, antigen-presenting remnants may still be confined inside the allograft. There is no literature available about RBC remnants in frozen bone allografts. However, a unit of fresh frozen plasma, containing fewer than $1 \times 10^8$ RBCs, is considered safe with respect to the risk of rhesus D immunization (2). This suggests that more RBC remnants were present in the used bone allograft in this case.

Little is known about RBC alloimmunization after organ transplantation. Presumably, this is not very common in organ-transplant patients due to the use of immunosuppressants. However, this is uncommon in bone-transplant cases, which may explain why alloimmunization did occur. This would suggest that alloimmunization occurs more often after bone-allograft transplantation. To our knowledge only seven cases of RBC alloimmunization after bone allografting have been described in the literature (3-6), all females. All of them were already of childbearing age, although prior pregnancies were denied. On the other hand, a study of 144 patients with transplanted cancellous bone chips did not show alloimmunization (7). However, the authors acknowledge that only small amounts of bone were transplanted.

Conclusion
We report here the first male patient who developed RBC alloantibodies after bone-allograft transplantation, providing evidence that transplantation of nonvascular tissue such as a bone allograft can cause primary RBC alloimmunization.

References