

Discriminating fetomaternal hemorrhage from maternal HbF-containing erythrocytes by dual-parameter flow cytometry.

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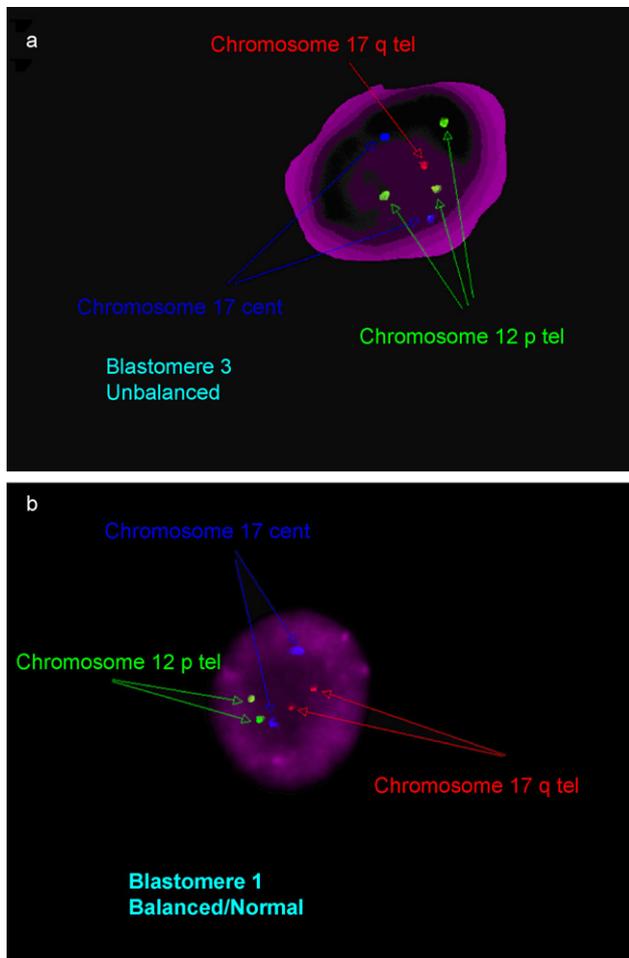


Fig. 1. (a) Multicolor FISH of blastomere 3 (embryo 2) of patient 1 displays an unbalanced chromosomal constitution reflected by two aqua signals of chromosome 17, one orange signal of long arm telomeric region of chromosome 17 (partial monosomy of chromosome 17) and three green signals of short arm telomeric region of chromosome 12 (partial trisomy of chromosome 12). (b) Multicolor FISH of blastomere 1 (embryo 4) of patient 1 displays a balanced chromosomal constitution, reflected by two aqua signals of chromosome 17, two orange signals of long arm telomeric region of chromosome 17 and two green signals of short arm telomeric region of chromosome 12. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

and has a key role in enabling the delivery of healthy babies.

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Discriminating fetomaternal hemorrhage from maternal HbF-containing erythrocytes by dual-parameter flow cytometry

Dear Editor,

We report a case of a pregnant women with a false-positive Kleihauer–Betke test and present a method to reliably discriminate fetomaternal hemorrhage from maternal HbF-containing erythrocytes.

A 37-year-old primigravida was admitted to our emergency room at 27+1 weeks of gestation after a blunt abdominal trauma. Upon admission she felt normal fetal movements without any contractions. There was no loss of blood or amniotic fluid. Physical examination showed no abnormalities. The fundal height was according to gestational age. The patient was admitted and closely monitored

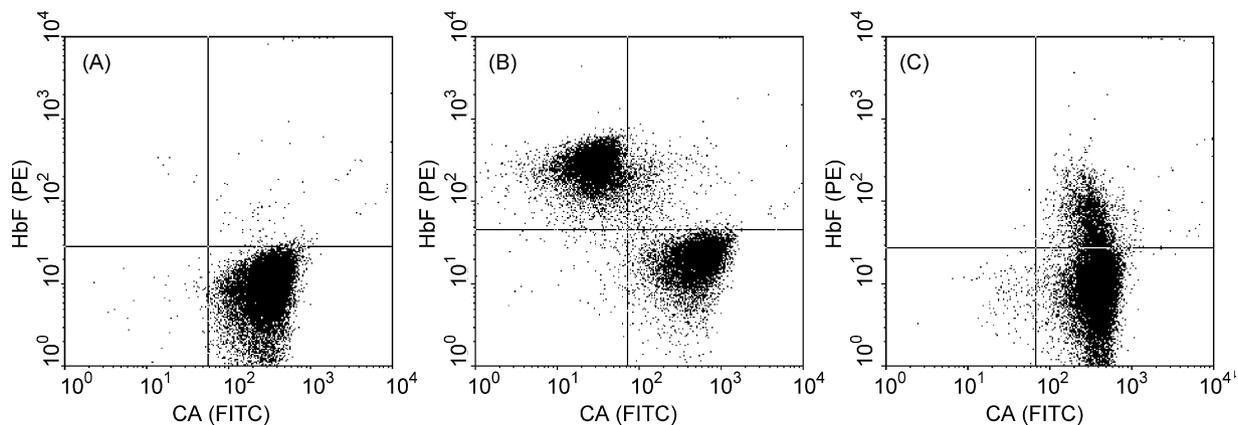


Fig. 1. Dual parameter (CA/HbF) flow cytometric analyses of erythrocytes. Cells are incubated with fluorescently-labeled antibodies against carbonic anhydrase (CA) and HbF. Erythrocytes with fluorescence intensities above the horizontal cut-off lines are designated HbF positive, erythrocytes to the right of the vertical cut-off line are CA positive: (A) normal adult control; (B) artificial sample consisting of adult blood and cord blood; and (C) blood sample of the patient described in this report. For interpretation of the results see text.

for 24 h. The fetal heart rate pattern was normal. Transabdominal ultrasound showed a vital fetus with an estimated weight according to gestational age. A Kleihauer–Betke test was performed and repeatedly showed 4% HbF-containing erythrocytes, suggesting a fetomaternal hemorrhage (FMH) of about 200 ml. Hemoglobin electrophoresis showed 2% HbF (reference range 0.1–1.5%), 96% HbA and 2% HbA₂.

To distinguish fetal erythrocytes from HbF-containing maternal erythrocytes, dual-parameter flow cytometry was performed using a commercially available kit (IQ-Products, Groningen, The Netherlands). This kit separates adult- from fetal-HbF-containing erythrocytes based on the expression level of carbonic anhydrase (CA). In an adult control sample a single CA-positive/HbF-negative population was present, representing adult erythrocytes containing HbA and CA (Fig. 1A). Upon addition of cord blood to that sample (Fig. 1B) a CA-negative/HbF-positive population of fetal cells appeared. The patient sample also contained two populations: a large population consisting of CA-positive/HbF-negative erythrocytes (95%; lower right quadrant, Fig. 1C) and a small population of CA-positive/HbF-positive cells (5%; upper right quadrant Fig. 1C). The first population consists of adult erythrocytes containing hemoglobin A, the second population are adult erythrocytes with hemoglobin F. No fetal erythrocytes could be detected in the patient sample (CA-negative/HbF-positive; upper left quadrant (Fig. 1C)). The initial readings of the Kleihauer–Betke slides were amended as uninterpretable due to presence of fetal hemoglobin of maternal origin. The patient was discharged with a preliminary diagnosis of presumed hereditary persistence of fetal hemoglobin (HPFH) Swiss type. Four months post partum blood was drawn from the mother and DNA analysis revealed the presence of the Xmn-I polymorphism (–158 C → T) of the G-gamma globin gene, known to be associated with elevated HbF expression in adult life [1].

Minute transfusions of fetal erythrocytes into the maternal circulation occur during normal pregnancy. However, larger FMH may have serious consequences, including isoimmunization, hemolytic disease of the newborn, fetal hypoxia and intra-uterine death. The Kleihauer–Betke test is a widespread procedure for assessment of FMH, but has well-recognized technical limitations and poor performance characteristics [2]. Moreover, hemoglobinopathies, thalassemias, anemias, HPFH, Xmn-I polymorphism (as in this report) and other conditions may be associated with elevated HbF levels in adult erythrocytes and thus may cause a false-positive Kleihauer–Betke test. Similarly, the flow cytometric assays described so far, while objective and accurate in quantitating FMH [3], cannot reliably discriminate between adult HbF-containing erythrocytes and fetal cells. The flow cytometric assay described here separates fetal erythrocytes from adult HbF-containing cells by using an anti-carbonic anhydrase-antibody as marker for adult erythrocytes.

Considering the diagnostic properties of the Kleihauer–Betke test and this flow-cytometric assay for detection of FMH we suggest a work-up of suspected FMH as follows. The Kleihauer–Betke test is used to screen for HbF-containing erythrocytes in patients suspected of FMH. If the test is negative, FMH is excluded. If positive, CA/HbF flow cytometry is used to discriminate between fetal erythrocytes and maternal HbF-containing cells. Fetal erythrocytes in the maternal circulation confirm the diagnosis FMH and the volume of fetal blood transfused can be calculated from the flow cytometric results. Detection of only adult HbF-containing cells excludes FMH and warrants further investigations into the cause of elevated HbF.

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Advanced cervical pregnancy: Diagnostic and management challenges

Dear Editors,

We would like to present a case of advanced cervical pregnancy and discuss the diagnostic and management options.

A 31-year-old, with one living child, three late miscarriages between 20 and 22 weeks, and two Caesarean sections at 26 and 29 weeks, presented to us at 13 weeks for booking.

A transabdominal and transvaginal ultrasound was suggestive of a cervical pregnancy but implantation in the uterine scar could not be entirely excluded. The Colour Doppler application was in keeping with cervical pregnancy. She had had some minor vaginal bleeding but was otherwise asymptomatic.

At 15 weeks of pregnancy, following an examination under anaesthesia during which a bimanual examination combined with transabdominal ultrasound was performed, a cervical ectopic was indeed confirmed with 1 cm of cervix below it, and an empty uterus.

After extensive counseling with regards to all the risks, a conservative approach was adopted as E.B was very keen to retain her fertility given her poor obstetric history. Thus,

after receiving 400_{mcg} of Mifepristone, she was started on methotrexate (1 mg/kg of body weight) with folinic acid administered on alternate days.

After four doses of methotrexate, a live cervical pregnancy persisted but the patient remained asymptomatic. However, in view of the advancing gestation and likelihood of significant bleeding, a decision was taken with the patient's consent for fetocide. This was followed by injection of 25 mg of methotrexate into the gestational sac.

The following day we proceeded to surgical evacuation of the pregnancy under ultrasound and laparoscopic guidance as she had begun to bleed. An interventional radiologist was available for emergency hypogastric artery embolization if profuse cervical bleeding was encountered. She received intra-operative broad spectrum antibiotics. The procedure was complicated by blood loss of approximately 2000 ml. Haemostasis was eventually achieved by insertion of a Sengstaken-Blakemore Tube (S-B Tube), and secured in place with a MacDonald cervical suture. She received in total 7 units of blood.

During the second postoperative day, she was started on intravenous Cefuroxime, Gentamycin and Metronidazole as there was clinical evidence of sepsis. The S-B tube was removed after 48 h. Her renal function which had become deranged during the immediate postoperative period improved, suggesting possible ureteric compression by the S-B tube. The cervical suture was removed on day 10.

Day 13, she was well for discharge. At her 6 weeks review, E.B continues to be well and is positive with regards to her experience.

Cervical pregnancy usually presents with vaginal bleeding in the first trimester. Our patient had remained mainly asymptomatic until the second trimester.

Confirming the diagnosis with ultrasonography alone becomes more difficult with advancing gestation [1]. It has been suggested that magnetic resonance imaging may be necessary to make an accurate diagnosis in such cases [2]. In our case, we used a combination of examination under anaesthesia with ultrasound guidance which was uncomplicated. Various treatment modalities, for cervical pregnancies, with varying success rates have been described in the literature.

In early gestation, systemic methotrexate has been used. Other methods with favourable outcomes are angiographic embolization of the uterine arteries, or ligation of descending branches of the uterine arteries with Shirodkar suture placement prior to evacuation of retained products of conception [3,4].

In other reported cases of cervical pregnancy at advanced gestation, laparotomy followed by hysterectomy was necessary [5]. We did not pursue these options because of our patient's strong desire to preserve her fertility.

The differential diagnosis of a scar pregnancy was initially considered bearing in mind her past obstetric history. However, diagnostic criteria such as discontinuity in