Prenatal diagnosis of \( \beta \)-thalassaemia by fetal red cell concentration with anti-AB serum\(^1\)

MARIO FURBETTA, CARLO VALENTI, ADA XIMENES,
ANTONELLA ANGIUS, TERESA TUVERI, PIERO FIORETTI, AND
ANTONIO CAO

From the Medical Diagnostic Center, Roma; 2nd Pediatric Clinic, University of Cagliari; and the
Department of Obstetrics and Gynecology, University of Cagliari, Sardinia, Italy

SUMMARY  Prenatal diagnosis by globin chain synthesis analysis on fetal blood samples obtained by
placental aspiration was performed in two pregnancies at risk for \( \beta \)-thalassaemia. Since both fathers
had blood group AB, and both mothers had group O, fetal red cell concentration from maternal-fetal
mixtures was obtained with the aid of anti-AB serum. With this approach it was possible to carry out
globin chain synthesis analysis with very small amounts of fetal blood. Heterozygous and
normal genotypes were ascertained and confirmed after birth.

In the last few years, prenatal diagnosis of inherited
haemoglobin disorders by fetal blood sampling and
globin chain synthesis analysis of fetal red cells has
been found to be feasible (Kan et al., 1975, 1977;
Alter et al., 1976; Alter and Nathan, 1978; Fair-
weather et al., 1978; Kan, 1978).

The elimination of the maternal contribution to
globin chain synthesis in mixed maternal-fetal blood
samples has been carried out in three ways: (1)
transfusion of the mother to suppress red cell
production (Alter et al., 1978; Fairweather et al.,
1978); (2) fetal red cell concentration with differential
agglutination, using anti-i serum (Kan et al., 1975,
1977); and (3) differential lysis of maternal cells with
the \( \beta \)-thalassaemia reaction (Alter et al.,
1976). Since high titre anti-i is rare, given certain
blood group combinations in the parents, fetal red
cell enrichment could be obtained with other anti-
bodies against blood group antigens (ABO, MNs, Rh
systems).

This paper reports the results of prenatal diagnosis
in 2 couples at risk for \( \beta \)-thalassaemia, carried out
by differential agglutination of fetal red cells with
anti-AB serum, as the fathers’ blood groups were
AB and the mothers’ O.

Subjects and methods

Two couples, one of Sardinian origin (case 1) and
the other from Ferrara (case 2), at risk for \( \beta^0 \) and
\( \beta^+ \)-thalassaemia, respectively, requested prenatal
diagnosis after genetic counselling. ABO blood
group determination showed the mother to be O
and the father AB in both couples. In case 2, there
was Rh (D) incompatibility, so anti-D gammaglobulin
was given 24 h after blood sampling.

Fetal blood sampling was carried out at 22 and 21
weeks’ gestation, respectively, after placental locali-
sation by ultrasound (Picker model 80L). Fetal
blood sampling was attempted twice in case 1. The
second time, a blood sample of 20\( \mu l \) with 10\% fetal
red cells was obtained. In case 2, three blood samples
of 16, 18, and 9\( \mu l \) containing 40, 7, and 7\% fetal
red cells, respectively, were drawn in the first attempt.

Previous experiments, carried out with a mixture of
heterozygous \( \beta \)-thalassaemia group O blood
samples and newborn group A or B blood samples at
varying concentrations, showed that even with
minimal newborn red cell concentration (less than
5\%) it was possible to obtain satisfactory agglutina-
tion using anti-AB serum, when it was not possible
with anti-i serum. It was found that this concentra-
tion procedure gave no modification of the \( \beta/\gamma \) ratio
(unpublished results).

The placental blood was incubated with \( 3 \)H
leucine according to the method of Kan et al. (1969).
Maternal venous blood, drawn before placental
aspiration, was incubated in the same way. The
placental samples were washed three times with
normal saline (0.9 NaCl). A red cell suspension at
20\% (v/v) in saline was prepared. A total of 2.5 vol
red cell preparation was mixed with 1 vol anti-AB

---

\(^1\)This work was supported by grants from the Assessorato
Igiene e Sanità della Regione Autonoma della Sardegna,
Sindacato Lavoratori Bancari and \(^2\)CNR contract number
78.004.658.06.

Received for publication 14 December 1978
Prenatal diagnosis of β-thalassaemia by fetal red cell concentration with anti-AB serum

serum (Ortho Diagnostic Inc., New Jersey, USA). The tubes were spun at 300 rpm for 2 min. The red cells were then suspended in 4 ml anti-AB serum diluted 1:50 in saline. This suspension was left to sediment for 20 min, after which the supernatant was aspirated. This procedure was repeated until the supernatant was clear. After this, the samples were tested with the Kleihauer stain.

The globin extraction and globin chain synthesis analysis were carried out according to the method of Kan et al. (1969).

Results

The samples obtained with anti-AB differential agglutination were composed of a nearly pure fetal red cell preparation (Fig. 1).

In case 1, the β/γ ratio was 0.038 (Fig. 2) and in case 2 it was 0.10; in the latter case the β/γ ratio after differential agglutination with anti-i was 0.11. These values are in the range found in heterozygous β-thalassaemia and normal mid-trimester fetuses, respectively (Table). Both diagnoses were confirmed by globin chain synthesis analysis on cord blood. ABO blood group determination showed both newborn babies to be group A.

Table  β/γ ratios in fetuses at risk for β-thalassaemia

<table>
<thead>
<tr>
<th>Case</th>
<th>β/γ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.038</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
</tr>
<tr>
<td>8 normal fetuses*</td>
<td>0.084-0.12</td>
</tr>
<tr>
<td>26 β-thalassaemia trait fetuses*</td>
<td>0.028-0.075</td>
</tr>
<tr>
<td>23 homozygous β-thalassaemia fetuses*</td>
<td>0.00†</td>
</tr>
</tbody>
</table>

*, unpublished results; †, in one case at risk for β*-thalassaemia a β/γ ratio of 0.05 was found.

Fig. 1  Kleihauer stain after differential agglutination with anti-AB serum.

Fig. 2  Chromatogram of a placental blood sample of case 1. ——— 3H leucine incorporation; — — absorbance at 280 nm. Unlabelled newborn and sickle cell trait haemolysates were added to the sample as carriers.
Discussion

The study of these two pregnancies at risk for β-thalassaemia showed that the concentration of fetal red cells from maternal-fetal mixtures, for prenatal diagnosis of β-thalassaemia, can be carried out in selected cases with anti-AB serum instead of anti-i serum.

Differential agglutination with anti-AB serum was possible even with minimal fetal red cell concentration in very small blood samples; this would not have been possible with anti-i serum (Kan et al., 1977). Anti-AB differential agglutination produces no modification in the β/γ ratio.

Complete blood group testing should be carried out in both members of a couple before attempting prenatal diagnosis of β-thalassaemia, in order to find genetic paternal-maternal blood group combinations which permit the use of antibodies other than anti-i for fetal red cell concentration.

References


Requests for reprints to Professor Antonio Cao, 2nd Pediatric Clinic, Via Porcell 1, 09100 Cagliari, Sardinia, Italy.
Prenatal diagnosis of beta-thalassaemia by fetal red cell concentration with anti-AB serum.

M Furbetta, C Valenti, A Ximenes, A Angius, T Tuveri, P Fioretti and A Cao

doi: 10.1136/jmg.16.5.366

Updated information and services can be found at:
[http://jmg.bmj.com/content/16/5/366](http://jmg.bmj.com/content/16/5/366)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to:
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to:
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)