# AMNIOTIC FLUID PROTEIN AND ANTENATAL DIAGNOSIS

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Between 10 weeks of pregnancy and term the bulk of the protein found in amniotic fluid is of serum type, dominated by albumin, IgG, transferrin, and α-lipoprotein. That this protein originates from the maternal rather than the fetal circulation has been demonstrated by the use of polymorphic markers as well as by the study of relative concentration with advancing gestation. However, proteins of high molecular weight such as β-lipoprotein, $\alpha_2$-macroglobulin and IgM, appear to be excluded from the fluid under normal circumstances[8].

The predominantly maternal origin of amniotic fluid protein severely restricts the usefulness of the fluid supernatant in antenatal diagnosis. The major exceptions to this statement are:

1. the widescale use of the feto-specific protein, α-fetoprotein (AFP), in the diagnosis of spina bifida, anencephaly and several other congenital malformations;

2. the growing use of acetylcholinesterase (AChE) as an ancillary tool in diagnosis of neural tube defects;

3. the titration of certain serine proteases in the antenatal diagnosis of cystic fibrosis of the pancreas;

4. the occasional analysis of lysosomal hydrolases in the diagnosis of specific inborn errors of metabolism.

*α-fetoprotein*

α-fetoprotein is a prototype oncofetal antigen, a protein present at high concentrations in early embryonic and fetal tissue, which largely disappears from adult tissues but reappears in the presence of certain types of tumour. It is found in human fetal sera from as early as six weeks of gestation.

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Concentrations rise quite rapidly, reaching a peak at the end of the first trimester, at which time levels of up to 3 to 4 mg/ml have been found, thus making it second only to albumin in terms of quantitative importance. Thereafter, although net AFP synthesis remains constant until about the 30th week of *in utero* life, the rapidly expanding fetal blood volume causes a steady decrease in concentration. At birth, the AFP level in cord serum from normal mature infants is between 10 and 150 μg/ml. Serum AFP concentration in newborn babies falls rapidly in the first few weeks of life and is usually not greatly different from the adult level by six months of age. AFP concentrations in normal adult serum lie between 2 and 20 ng/ml, about 1/100,000 times the concentration found in the 13-week fetus. Concentrations comparable to those found in fetal serum have been reported in fetal cerebrospinal fluid. AFP can be detected in normal human amniotic fluid throughout gestation, at about 1/100 of the level found in fetal serum at the corresponding gestation. It is also detectable in fetal urine throughout gestation at levels high enough to suggest that this is a major origin of amniotic fluid AFP[1].

The use of AFP in the early diagnosis of fetal neural tube defects was discovered in 1972[2]. The rationale for its usage was the idea that in malformations where there might be communication between the fetal serum or cerebrospinal fluid and external surfaces, a feto-specific protein should provide a valuable marker. This has indeed turned out to be the case, and AFP is now widely used not only on amniotic fluids from pregnancies with an increased risk of neural tube defect, but often routinely on all amniotic fluid samples. It must be emphasized that since AFP is not the product of a malfunctioning gene or a defective chromosome, it will not have either the specificity or sensitivity normally required in antenatal diagnosis.

The most comprehensive analysis of the precision of amniotic fluid AFP in the diagnosis of open neural tube defects comes from the Second Report of the UK Collaborative Study[6]. This study combined data from 17 individual laboratories covering 385 pregnancies where the outcome was a fetal neural tube defect (including 222 with anencephaly, 152 with spina bifida of which 123 were known to have open lesions, and 11 with encephalocele), and on 13,105 pregnancies where there was no neural tube defect. To obviate the systematic differences found between laboratories in the reported AFP concentration on identical samples, AFP values were expressed in multiples of the median value for a given week of gestation. The best resolution between normal and abnormal pregnancies was found to be achieved by raising the cut-off as gestation advanced. As shown in tab. 1, a cut-off of 2.5 multiples of the median (MoM) at 13-15 weeks, 3.0 MoM at 16-18 weeks, 3.5 MoM at 19-21 weeks, and 4.0 MoM at 22-24 weeks allowed a sensitivity of 97.6% for open spina bifida and 98.2% for anencephaly. The overall false positive rate at these cut-offs was 0.79%. It was found that if higher cut-offs were chosen the sensitivity for open neural tube defects declined, but that there were fewer false positives.

The overall false positive rate of 0.79% is somewhat misleading. To a large extent this arises from the fact that pregnancies not associated with neural tube defects were considered as 'unaffected'. These pregnancies included a number of malformations known to be variably associated with ele-

vated AFP, as well as some that ended in miscarriage, and others where fluids were contaminated with both maternal and fetal blood. When spontaneous abortions and other fetal abnormalities were removed from the pooled data, the false positive rate reduced to 0.53%. Where only clear amniotic fluids were considered, the false positive rate was 0.30% (tab. 2). In neither of these latter cases was the sensitivity of the system affected. While it is possible to consider a false positive rate independently of other fetal abnormalities, it is not practical to discard blood-stained amniotic fluids. Thus, the conclusion of the UK Collaborative Study was that if its system of rising cut-offs with advancing gestational age were used, about 98% of neural tube defects would be detected with a false positive rate of around 0.5%.

In addition to marking the presence of anencephaly and spina bifida, raised amniotic fluid AFP concentrations are found to be associated with a variety of other fetal abnormalities. As yet the accumulated data relating to these conditions are somewhat sparse. Many of the abnormalities listed in tab. 3 are rare and the opportunity for making observations is therefore limited. Several reports have referred only to increased amniotic fluid AFP in the third trimester of pregnancy, and there is no certainty that the same situation would hold for those weeks of the second trimester when the majority of amniocenteses are performed. Even when adequate data have been collected before the 20th week of pregnancy, it is still apparent that some conditions are not invariably associated with increased amniotic fluid AFP, and that this may depend *inter alia* on the size, nature and position of the lesion[1].

| gestation (weeks) | cut-off (MoM) | % of detected cases | |
|---|---|---|---|
| | | anencephaly | open spina bifida |
| 13-15 | 2.5 | 100 | 96 |
| 16-18 | 3.0 | 99 | 99 |
| 19-21 | 3.5 | 99 | 95 |
| 22-24 | 4.0 | 94 | 100 |
| all | as above | 98.2 (218/222) | 97.6 (120/123) |

Tab. 1 - Amniotic fluid AFP: detection efficiency for neural tube defects. (Adapted from the Second Report of the UK Collaborative Study[6]).

| type of sample | no. | % |
|---|---|---|
| all samples | 103/13,105 | 0.79 |
| all normal samples | 68/12,804 | 0.53 |
| all normal clear samples | 35/11,625 | 0.30 |

Tab. 2 - False positive rates in amniotic fluid AFP assay. (Adapted from the Second Report of the UK Collaborative Study[6]).

| | |
|---|---|
| anencephaly | open spina bifida |
| intrauterine death | congenital nephrosis |
| Meckel's syndrome | exomphalos |
| gastroschisis | exstrophy of the cloaca |
| oesophageal atresia | duodenal atresia |
| fetal teratoma | hydrocephalus |
| Turner's syndrome | congenital skin defects |
| fetus papyraceous | osteogenesis imperfecta |
| Fallot's tetralogy | pilonidal sinus |
| nuchal bleb | Rh-isoimmunization |

Tab. 3 - Fetal abnormalities in which elevated concentrations of amniotic fluid AFP have been reported. (Adapted from BROCK [1]).

The main technical problem encountered in measuring amniotic fluid AFP occurs when the sample is extensively blood-stained. Contamination by maternal blood, a not infrequent occurrence during amniocentesis, is unimportant since maternal serum AFP levels in early pregnancy are only 1/100 to 1/500 of those in the fluid itself. But difficulties arise when fetal blood is found in the amniotic fluid, for fetal serum AFP concentrations are 100 to 200 fold higher than those in the fluid itself. Thus, comparatively moderate contributions of fetal blood can seriously distort amniotic fluid AFP concentrations and may occasionally mimic the levels associated with neural tube defects. It is probable that several of the 'false-positive' diagnoses recorded in the literature are due to unrecognized AFP contributions from fetal blood. Ideally the origin of any blood in any amniotic fluid with a raised AFP value should be ascertained before any decision is made about whether the pregnancy is abnormal. Though there are methods of calculating the expected contribution of AFP from fetal plasma to the total AFP measured in an amniotic fluid, such estimates depend on a number of variables and cannot be viewed with any confidence. It is probably a better practice to endeavour to perform a second amniocentesis after an appropriate period of time[1]. Recently, however, the advent of AChE measurement has greatly facilitated the interpretation of fetal blood-stained amniotic fluids.

*Acetylcholinesterase*

Acetylcholinesterase (AChE) belongs to the group of cholinesterase enzymes, all of which hydrolyse choline esters faster than other esters. Most human tissues contain a variety of cholinesterase isoenzymes, dominated by the non-specific pseudocholinesterases. Brain and neural tissue, however, are enriched in AChE. Most of it is membrane-bound, but one AChE isoenzyme appears to be secreted into the cerebrospinal fluid. Any analysis of AChE must therefore distinguish it from the more abundant pseudocholinesterases. In quantitative spectrophotometric assays this is achieved by the use of inhibitors, of which 'lysivane' that suppresses mainly pseudocholinesterases and 'BW284C51' that suppresses AChE are the most commonly employed. Unfor-

tunately neither of these inhibitors is absolutely specific and it is therefore difficult to sort out AChE activity from total activity of the other cholinesterases.

To a very large extent this problem is solved by the use of polyacrylamide gel electrophoresis[7]. Analysis of a small aliquot of amniotic fluid allows AChE to be distinguished from pseudocholinesterases on the basis of differential mobility (fig. 1). Furthermore, the precise nature of the bands seen on polyacrylamide after staining for cholinesterase activity can be confirmed by the use of the inhibitors lysivane and BW284C51. In general, amniotic fluids from normal pregnancies reveal a single comparatively slow-moving band which can be suppressed by lysivane and is thought to be pseudocholinesterase. In the presence of an open neural tube defect an additional faster-moving band becomes visible which can be inhibited by BW284C51 and therefore presumed to be AChE. Thus, in the polyacrylamide gel electrophoretic procedure both mobility and susceptibility to specific inhibitors are used to establish the nature of the isoenzymes.

Studies assessing the potential of gel electrophoretic analysis of AChE have given impressive results. In many respects this qualitative test appears superior to the quantitative amniotic fluid AFP assay, both in resolving 'false negative' cases of open neural tube defect and 'false positive' normal pregnancies. In general, however, AChE bands also appear in amniotic fluids from pregnancies associated with other fetal abnormalities in which AFP is raised (tab. 4). The main exception is congenital nephrosis, where AChE bands are not observed even with grossly elevated AFP concentrations.

These encouraging results have prompted a collaborative study[3] aimed at assessing the value of AChE as a secondary test in the diagnosis of anencephaly and open spina bifida. In this investigation AChE measurement by the

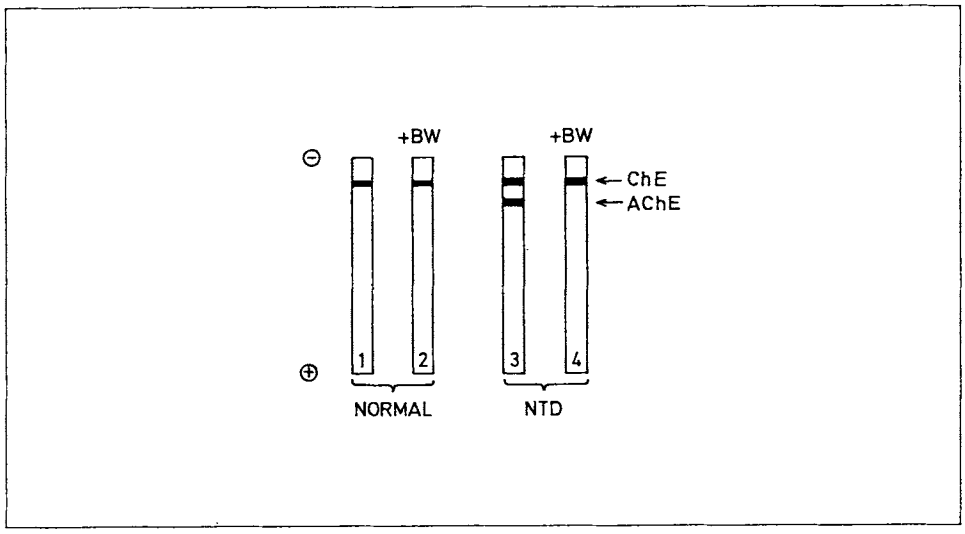


Fig. 1 - Diagrammatic representation of cholinesterase band patterns after polyacrylamide gel electrophoresis of amniotic fluid. Gels 1 and 2 from normal pregnancies, gels 3 and 4 from neural tube defect pregnancies. In gels 2 and 4 the inhibitor BW284C51 has been used.

| outcome of pregnancy | no. | no. (%) with positive AChE |
|---|---|---|
| anencephaly | 478 | 476 (99.6) |
| open spina bifida | 335 | 333 (99.4) |
| exomphalos | 63 | 47 (75) |
| congenital nephrosis | 11 | 0 (0) |
| Turner's syndrome | 4 | 4 (100) |
| fetal teratoma | 3 | 1 (33) |
| miscarriage | 73 | 34 (47) |
| apparently normal | 125 | 8 (6) |

Tab. 4 · Numbers of pregnancies with positive amniotic fluid AFP results and positive AChE results. (Adapted from the Collaborative Acetylcholinesterase Study[3]).

gel test was made on 1,092 women, all of whom had a positive amniotic fluid AFP result. Of pregnancies with anencephaly, 99.6% (476/478) revealed an AChE band, while of those with open spina bifida 99.4% (333/335) were likewise positive. Only 6% (8/125) of pregnancies, which did not end in a miscarriage and which were not associated with a serious fetal malformation, yielded positive AChE results (tab. 4). In essence this means that a woman who has an elevated amniotic fluid AFP concentration and also a positive AChE test is 16 times as likely to be carrying a serious fetal malformation as the woman with only an elevated AFP result.

An unanswered question concerning AChE testing is the rate of false positives in unselected samples. It is assumed that the presence of AChE in amniotic fluid is less influenced by the presence of fetal blood than that of AFP. This has yet to be rigorously tested in prospective studies. There are also problems associated with a qualitative 'yes/no' type of test which bear a closer examination. Until more is known about the specific problems associated with AChE testing, it would be unwise to use it as a primary method for the diagnosis of fetal neural tube defects.

*Serine proteases and cystic fibrosis*

Some years ago, NADLER et al.[5] observed that when protease levels in plasma from cystic fibrosis patients were measured with the artificial substrate 4-methyl-umbelliferyl-guanidino-benzoate (MUGB), significantly depressed values were found. Intermediate values were observed in obligate heterozygotes. The mode of determination of MUGB protease was titration rather than rate assay, and appeared to detect a 'trypsin-like' activity, thought to be associated with serine proteases. The differences between titres of MUGB activity in plasma or serum of homozygotes, heterozygotes and normals could be enhanced by the use of a benzamidine inhibitor, which appeared to eliminate the contribution of non-trypsin-like proteases. Similar results were found in cultured skin fibroblasts and in cultured amniotic fluid cells.

Despite the observation of MUGB protease activity in cultured amniotic fluid cells, the use of this enzyme in early antenatal diagnosis of cystic fibro-

sis has so far been restricted to amniotic fluid supernatant. It has been observed that MUGB titre is fairly constant for a variety of amniotic fluids at different weeks of gestation, and is substantially altered when the fetus has a neural tube defect or chromosome abnormality. NADLER et al.[9] carried out MUGB estimations on 6 amniotic fluids from pregnancies where amniocentesis had been carried out for other reasons but where the liveborn child had subsequently been shown to have cystic fibrosis. All 6 fluids had titres more than two standard deviations below the mean value. This work has now been extended to the prospective diagnosis of cystic fibrosis. NADLER and WALSH[4] have reported the monitoring of 13 pregnancies in which the mother had had a previous affected child, and which subsequently led to three affected infants and 10 normals, as judged by the criterion of sweat test. In each of the three affected cases the MUGB values lay between 2 and 3 standard deviations below the mean (fig. 2). These authors have also suggested that the reliability of this form of antenatal diagnosis can be improved by examining the stability of the proteases and their electrophoretic migration pattern in amniotic fluid. Though these results must be seen as essentially preliminary, they offer at present the best hope of a reliable method for the early detection of the fetus with cystic fibrosis.

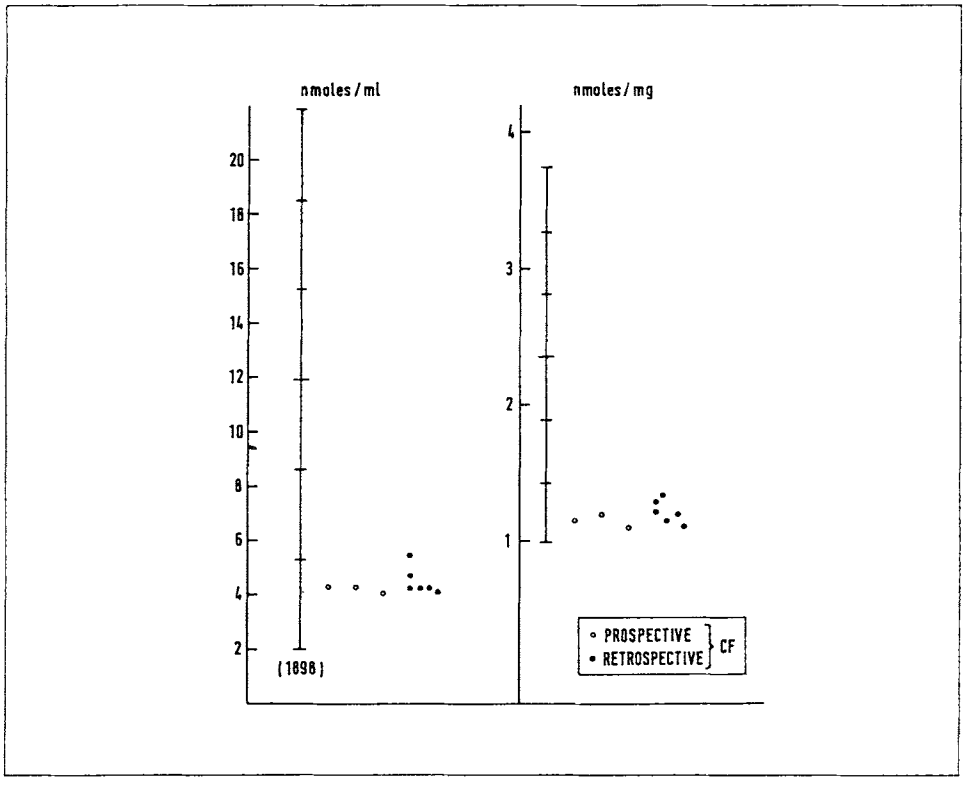


Fig. 2 - The use of MUGB protease titre in the prenatal diagnosis of cystic fibrosis. The solid bars represent the mean ± standard deviations, and the circles are values obtained in at-risk pregnancies. (From NADLER and WALSH[4]).

| disorder | enzyme deficiency |
|---|---|
| generalized gangliosidosis | β-galactosidase |
| Tay-Sachs disease | hexosaminidase A |
| Sandhoff's disease | hexosaminidase A and B |
| Fabry's disease | β-galactosidase |
| Krabbe's disease | cerebroside β-galactosidase |
| Niemann-Pick disease | sphingomyelinase |
| metachromatic leukodystrophy | arylsulphatase A |
| fucosidosis | α-fucosidase |
| Hunter's syndrome | iduronate sulphatase |
| I-cell disease | lysosomal hydrolases |

Tab. 5 · Some lysosomal enzymes whose activities in amniotic fluid supernatant have been used in antenatal diagnosis. (Adapted from SUTCLIFFE[8]).

*Lysosomal hydrolases*

A large number of different enzymes have been detected and measured in amniotic fluid supernatant. In general there is a wide normal range of specific activity for each enzyme throughout pregnancy. Maximum activities of individual enzymes occur at different stages of pregnancy, and are often unrelated to total protein concentration. For this reason most workers view the measurement of enzyme activity in amniotic fluid supernatant with considerable suspicion and regard it as a last-resort method of antenatal diagnosis.

The only situation in which amniotic fluid supernatant enzyme activity may be used in detection of affected fetuses is for those lysosomal enzymes associated with certain specific inborn errors of metabolism[8]. A list of these is shown in tab. 5. Though in most cases the level of enzyme in the amniotic fluid surrounding an affected fetus has been substantially depressed, this is not invariably the case and erroneous diagnoses may occur. This is particularly true if amniotic fluid is contaminated by maternal blood, thus conferring spuriously elevated values to the amniotic fluid. It must therefore be emphasized that this form of antenatal diagnosis should only be contemplated if there is an irreversible failure in the culture of amniotic fluid cells.

REFERENCES

1. BROCK D. J. H.: Feto-specific proteins in prenatal diagnosis · Molec. Aspects Med. *3*, 431, 1980.

2. BROCK D. J. H., SUTCLIFFE R. G.: Alphafetoprotein in the antenatal diagnosis of anencephaly and spina bifida · Lancet *ii*, 197, 1972.

3. COLLABORATIVE Acetylcholinesterase Study · Lancet *ii*, 321, 1981.

4. NADLER H. L., WALSH M. M. J.: Intrauterine detection of cystic fibrosis · Pediatrics *66*, 690, 1980.

5. RAO G. J. S., WALSH-PLATT M., NADLER H. L.: Reaction of MUGB with proteases in plasma of patients with cystic fibrosis · Enzyme *23*, 314, 1978.

6. SECOND Report of the UK Collaborative Study on Alphafetoprotein in relation to Neural Tube Defects - Lancet *ii*, 651, 1979.

7. SMITH A. D., WALD N. J., CUCKLE H. S., STIRRAT G. M., BOBROW M., LAGERCRANTZ H.: Amniotic fluid cholinesterase as a possible diagnostic test for neural tube defects in early pregnancy - Lancet *i*, 685, 1979.

8. SUTCLIFFE R. G.: Protein in human amniotic fluid. In: SANDLER M. (Ed.): Amniotic fluid and its clinical significance. Marcel Dekker, Inc., New York, 1981; p. 129.

9. WALSH M. M. J., RAO G. J. S., NADLER H. L.: Reaction of MUGB with proteases in human amniotic fluid - Pediat. Res. *14*, 353, 1980.

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