Testing for osteogenesis imperfecta in cases of suspected non-accidental injury

A Marlowe, M G Pepin, P H Byers

To evaluate if laboratory testing for osteogenesis imperfecta (OI) identifies children unrecognised by clinical examination in instances where non-accidental injury (NAI) is suspected as the likely cause of fracture, we carried out a retrospective review of available medical records and biochemical test results from 262 patients. Cultured fibroblasts were received for biochemical testing for OI from children in whom the diagnosis of NAI was suspected. Eleven of the samples had alterations in the amount or structure of type I collagen synthesised, consistent with the diagnosis of OI, and in 11 others we could not exclude OI. Referring physicians correctly identified children with OI in six of the 11 instances established by biochemical studies, did not identify OI by clinical examination in three, and there was inadequate clinical information to know in two others. Biochemical testing was inconclusive in 11 infants in whom the diagnosis of OI could not be excluded, none of whom were thought to be affected by the referring clinicians. Four children believed to have OI by clinical examination had normal biochemical studies, a false positive clinical diagnosis attributed, in large part, to the use of scleral hue (a feature that is age dependent) as a major diagnostic criterion.

Given the inability to identify all children with OI by clinical examination in situations of suspected NAI, laboratory testing for OI (and other genetic predispositions for fractures) is a valuable adjunct in discerning the basis for fractures and may identify a small group of children with previously undiagnosed OI.

Non-accidental injury (NAI) is a major public health concern that affects an estimated 800 000 children each year (National Child Abuse and Neglect Data System, 1999, http://www.acf.dhhs.gov/programs/cb/publications), and involves their parents and family members, physicians, nurses, social workers, and others who participate in the care and evaluation of these children. Health care providers in the United States are compelled by federal law (Child Abuse Prevention and Treatment Act, 1974) to report suspected cases of child abuse. Although abuse is common, it may be difficult to discern the true cause of an injury and it is estimated that approximately 7% of children who have signs suggestive of abuse actually have an underlying medical condition that explains their injuries. Some of the more common genetic conditions that have clinical features which share features of NAI include osteogenesis imperfecta (OI), several types of Ehlers-Danlos syndrome (EDS), and glutaric acidemia type I. In its milder forms, OI may present simply as unexplained bone fractures in childhood.

When children present to the accident and emergency department or clinic with unexplained fractures, they and their parents are usually referred to Child Protective Services (CPS) for at least a preliminary evaluation. Depending on the results of this evaluation, infants and parents can be separated for anywhere from a few minutes to weeks or months, until the investigation is terminated. If the child who is separated from the family is shown subsequently to have OI, for example, the separation can have devastating effects on the family and their relations to medical and other health care or social service personnel. Conversely, parents who have abused their child may claim that the child has OI. In some instances, the return of previously abused children to the family can lead to further injury and even death.

OI is a genetic disorder that occurs in approximately 1:10 000 births. NAI with fractures occurs in approximately 24:10 000 children (National Child Abuse and Neglect Data System, 1999) in the birth to 3 year age range, so that a child is about 24 times more likely to have broken bones secondary to abuse than to OI. The diagnosis of OI depends on obtaining a careful family history, review of the x rays, and physical examination in which characteristic features (for example, blue sclerae, short stature, fractures, bone deformity, dentinogenesis imperfecta, and hearing loss, many of which have age dependent penetrance) are sought or identified. To recognise children with OI among those with fractures requires that physicians are familiar with OI as an entity and know how to make the clinical diagnosis. Often there is no family history of OI, and radiographic studies may be equivocal. The clinical differentiation of OI and NAI may be difficult. Fractures in various stages of healing can be seen in both, there may be a family history of fracture because of abuse, pale blue sclerae (one of the commonest features mentioned in examinations) are normal in young children, and some features of OI, such as DI and hearing loss, are not apparent in infancy.

The biochemical bases of most forms of OI were identified in the 1970s and shown to alter the amount or structure of type I collagen, the major protein of bone, synthesised. The first mutations were identified in the early 1980s and since then a few hundred mutations in the two type I collagen genes, COL1A1 and COL1A2, have been described (Database of Human Type I and Type III Collagen Mutations; http://www.ie.ac.uk/genetics/collagen/). These studies provided the basis for the biochemical and molecular genetic testing that is now available to assist in the diagnosis of OI. These studies take two forms. In the first, skin fibroblasts are grown and the amount and structure of the type I procollagen molecules synthesised by the cells is measured. This approach identifies almost 90% of subjects known to have OI.

Abbreviations: OI, osteogenesis imperfecta; NAI, non-accidental injury; EDS, Ehlers-Danlos syndrome; CPS, Child Protective Services; DI, dentinogenesis imperfecta
approach, the coding regions of the two genes (COL1A1 and COL1A2) that encode the chains of type I procollagen are screened for pathogenic sequence alterations, a strategy that also identifies about 90% of subjects known to have OI.

Currently, we study approximately 600 specimens each year from adults and children in whom the diagnosis of OI is being considered. About 10% of these tests are ordered in instances in which NAI is suspected, but OI is considered or cannot be excluded (fig 1). To determine the efficiency with which these studies identified children with OI among those in whom abuse was considered, we reviewed the clinical information provided for 262 samples in the context of the results of our biochemical studies. Cells from 11 of these infants had abnormalities consistent with OI and in 11 others the diagnosis could not be excluded. These findings confirm that these analyses have a place in the evaluation of children in whom the diagnosis of OI cannot be excluded on the basis of physical examination alone. Given the sensitivity of collagen screening and the relative frequencies of OI and NAI, it appears that only a small proportion of children with OI will be missed by this approach.

SUBJECTS AND METHODS

Clinical evaluation and data collection

We reviewed the clinical data provided to us about all subjects with a diagnosis of OI, possible OI, or fractures of unknown origin whose cells were sent to us to examine collagen synthesis and structure from 1 January 1998 to 28 February 2001 (n=1857). We identified those in whom abuse (non-accidental injury, NAI) was being considered to explain fractures; 263 such children were identified, all but seven under the age of 3 years. The mean age of the infants studied was 9.4 months with a median age of 6.0 months (fig 2). In one instance cells submitted did not grow. Studies of collagen synthesis and structure were performed in the other 262 samples. The clinical notes provided with samples from 138 of the 262 children contained sufficient detail to allow us to evaluate independently the likelihood that the subjects had OI using clinical criteria alone. To be included in this subset of 138 infants, the notes usually had to include a description of the fractures, notation of a family history of OI or frequent fractures, colour of sclerae, presence or absence of Wormian bones, bowing of extremities, osteopenia, and measurement of stature. Some descriptions that did not have all items in place were included if they had a physical examination with a listing of pertinent positives and/or negatives. Additional data collected from the notes provided included involvement of CPS, statements as to probable diagnosis, and the reasons given for testing.

Biochemical studies

Cultured fibroblasts were sent to us from the referring hospital or were grown in our laboratory from skin biopsies, as previously described. Collagenous proteins were labelled and analysed by polyacrylamide gel electrophoresis as previously described. The resulting autoradiographs were analysed for the amounts and mobilities of the chains of type I procollagen and collagen.

Characteristically, cells from subjects with OI type I make about half the normal amount of type I procollagen, but the chains have normal electrophoretic mobilities. In contrast, cells from subjects with OI type IV and many forms of OI type III make normal amounts of type I procollagen but the electrophoretic mobilities of the chains of type I procollagen are abnormal and the efficiency of secretion of the abnormal molecules is reduced.

![Figure 1](http://jmg.bmj.com/)

**Figure 1** Number of samples submitted for biochemical tests for osteogenesis imperfecta to the Collagen Diagnostic Laboratory at the University of Washington, 1990-2000. The number of samples submitted each year is indicated by the height of the white bar and the black bar indicates those submitted for an evaluation when abuse was also considered.

![Figure 2](http://jmg.bmj.com/)

**Figure 2** Age distribution in months of children for whom biochemical studies of cultured fibroblasts were completed. All samples studied (open circles, age data available for 238 of the total of 262 children); samples from those with adequate clinical histories for independent analysis (filled squares, age data available for 135 of the 138 children). Seven children with ages greater than 36 months are not included in this figure.
gene were amplified and cleaved with Mnl from cultured cells and the regions surrounding a polymorphic site in the 3′ untranslated region (3′ UTR) of the COL1A1 gene were amplified and cleaved with Mnl.24 When heterozygous, the two alleles were assayed in cDNA synthesised from mRNA by reverse transcription and amplification of the product by the polymerase chain reaction (RT-PCR) followed by digestion of the product with the same enzyme.24

RESULTS
Biochemical studies of collagen production and structure
In our studies of these 262 infants and children, we identified 11 in whom the diagnosis of OI was clear. Among these, cells from six synthesised about half the normal amount of type I procollagen, consistent with the diagnosis of OI type I. Cells from the remaining five synthesised some abnormal molecules, consistent with the diagnosis of OI type IV or OI type III. In another 11 we could not exclude OI, usually because we could not be sure if the amount of type I procollagen synthesised was in the normal range. In these instances, we tried to determine if both COL1A1 alleles were expressed in equal amounts, the polymorphic marker used (an Mnl site in the 3′ untranslated region) was homozygous in genomic DNA and thus non-informative.

Clinical correlations
The subgroup of 138 well described infants included nine of the 11 infants in whom we identified OI (six with biochemical findings of OI type I and three with the abnormal findings seen in subjects with OI type III or IV), six of the 11 in whom we could not exclude OI, and 123 in whom studies were normal (fig 3). The referring physicians, 90% of whom had training in clinical genetics, suspected that the children had OI in 10 of these 138 infants. They had designated six of the nine we showed to have OI as affected and thought that four of the 123 for whom normal results were obtained also had OI (table 1). In our review of the data provided for these latter four patients, the diagnosis of OI was suspected on the basis of fractures or “blue tinged” sclerae in three of four. In one instance, the basis of the diagnosis was unclear. Blue sclerae were noted in almost half of the 138 children and are recognised as an age dependent normal finding.23 24

For this group of 138 patients we could find no combination of additional clinical signs (for example, blue sclerae, short stature), associated history (family history or history of injury), or radiological features (nature and location of the fractures or Wormian or bowed bones), which, when combined with fractures, discriminated all affected infants from those we determined to be unaffected (table 2). In part, this may have been because we had incomplete clinical information and did not have x rays to review for most infants.

One unanticipated feature of the group we studied was the higher proportion (23/138, 16.7%) of infants born at 36 weeks’ gestation or less when compared to the US norm (9.0%, χ² = 7.67, p=0.01) (US Vital Statistics (http://www.cdc.gov/nchs/data)). Twelve were born before 32 weeks’ gestation and five had some degree of developmental impairment. Six were part of multiple births. Cells from one infant born at 34 weeks’ gestation synthesised somewhat less type I collagen than control cells and we were unable to exclude the diagnosis of OI type I. Cells from the rest synthesised normal amounts of type I collagen. The overall proportion of twins in this group (11/138, 7.9%) was greater than the expected 2.8% in the US population (χ² = 17.68, p=0.001) (US Vital Statistics (http://www.cdc.gov/nchs/data)).

Analysis of expression of COL1A1 alleles
In some cases there was a decreased amount of type I procollagen produced, but not enough of a decrease to allow us to confirm a diagnosis of OI. In these instances, DNA was isolated from cultured cells and the regions surrounding a polymorphic Mnl site in the 3′ untranslated region (3′ UTR) of the COL1A1 gene were amplified and cleaved with Mnl.24 When heterozygous, the two alleles were assayed in cDNA synthesised from mRNA by reverse transcription and amplification of the product by the polymerase chain reaction (RT-PCR) followed by digestion of the product with the same enzyme.24

Table 2 Clinical features and findings of nine subjects (with adequate clinical documentation) identified with abnormal type I procollagen

<table>
<thead>
<tr>
<th>Age*</th>
<th>Sex</th>
<th>Race †</th>
<th>Family history</th>
<th>Sclerae blue/grey</th>
<th>Bone phenotype</th>
<th>OI type</th>
<th>Diagnosis suspected‡</th>
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*Age in months.  †U, unspecified; W, white; H, Hispanic; A, African-American. ‡NS, not specified.
www.cdc.gov/nchs/data), and accounted for most of the pre-
maturity in this group so that twinning and prematurity were
not independent variables.

In the group of children whose cells made normal collagens,
36 of the 138 (26%) had clear evidence of additional trauma.
There were four who had died, one who was found
abandoned, 11 who had intracranial haemorrhages, five who
had retinal haemorrhages, and 14 who had bruises, burns,
bites, lacerations, or clear evidence of sexual abuse. For an
additional eight children, there had either been previous abuse
of children in the family or fractures stopped once the child
was removed from the home. This compares to one bloody
nose in the OI positive group and one laceration in the mouth
of one of the equivocal subjects.

Pattern of referral for testing
Of the 138 infants for whom we had the most complete data,
103 were known to have been referred to Child Protective
Services (CPS) and the data were incomplete for the other 35.
The court ordered testing for 21 of the 103; in two of them we
could not exclude OI. For an additional 24, the court was
involved in facilitating testing, although we were unsure of
the exact nature of the court’s role; none had OI. Among the
remaining 58 who had been referred to CPS, seven had OI and
in four we could not exclude the diagnosis. The two other
children in whom we made the diagnosis of OI were among
the 35 for whom we did not know if CPS was involved.

DISCUSSION
In our retrospective study, we identified 11 of the 262 infants
(4.2%) at risk to have NAI as affected with OI and could not
exclude the diagnosis in an equal number. Six of the 11 with
OI were considered to be affected by the referring physicians,
three were considered “possibly” affected, and there was no
information for the other two. Among those for whom
biochemical studies were equivocal, none was thought by the
referring physicians to have OI on clinical grounds.

Given the prevalences of OI and NAI in the USA, if children
with OI and those thought to have been intentionally injured
were combined into a single group, approximately 2-5% of the
total would be expected to have OI, approximately the
proportion we identified here and in our previous, smaller
study.3 The fact that the proportion of children with OI among
the total is similar to that expected if there were no
preliminary screening on clinical grounds suggests two
things. First, given that genetic testing was requested for
infants in whom the diagnosis of OI was suspected on clinical
grounds, it appears that physicians are compelled to request
laboratory confirmation of the OI diagnosis in instances of
possible non-accidental injury. Second, it is likely that in the
population of children with unexplained fractures, it is
difficult to distinguish those with OI on clinical grounds alone
in a subset of children, probably because of the age dependent
expression of clinical signs like dentinogenesis imperfecta,
hearing loss, bone deformity, and short stature. Thus, despite
our previous conclusion that clinical examination is likely to
provide a sufficient screening tool,21 it now seems clear that
some children with OI are not ascertained easily by examina-
tion alone and that laboratory testing can be an important
element in their identification.

These tests are efficient at identifying those with OI type I
and OI type IV, but less so those with OI type III, either because
the mutations do not alter protein or because the mutations
are in other genes.22 Because the x ray features of OI type III
should be readily recognisable in the 0-3 year age group and
because those children are readily identifiable by clinical
examination, it is children with OI type I or OI type IV that are
most likely to be overlooked by examination in the heat of the
moment when NAI is considered as the most likely source of
the injury. If we assume that the ability to detect biochemical
abnormalities in cells from these children is 90% and we iden-
tified 11 affected children, then we would have failed to detect
one child (or two, if the number “detected” is considered to be
22 instead of 11). Thus the chance that the 240 infants in this
study who had normal studies would have OI should be about
1% or less.

For reasons that are not clear, assessment of unexplained
fractures has evolved to revolve around the dichotomy of OI
and abuse. There are other predisposing genetic factors for
bone fragility and fracture that include hypophosphatasia and
related disorders, disorders of vitamin D metabolism,27-30 and
disorders of copper metabolism such as Menkes disease.2
To name only three additional genetic disorders.

The exclusion of OI as a contributing factor does not exclude
other factors, although the a priori likelihood of their involve-
ment is not high, because in most instances the other genetic
predispositions to fracture are less common than OI.

The relatively high proportion of premature infants in this
study reawakens the discussion of other factors that could
predispose to fracture in children. Prematurity contributes to
fracture predisposition among infants with birth weights less
out, perhaps because we did not enrol them in the 32-41, but
most of these fractures occur in the hospital. Only four infants
in our study population were born before 30 weeks’ gestation
suggesting that the prolonged hospitalisation and nutritional
concerns of the very premature were limited in this instance.
The increased risk for fracture in the premature does not seem
to be sustained into childhood. In studies of children under
the age of 5 who presented to an emergency room because of
fractures, the incidence of prematurity was not higher than
seen in children presenting for other reasons who did not have
fractures.29 Premature infants, particularly those with develop-
mental and medical concerns, may be members of a popu-
lation of “disabled” children who are at greater risk for
non-accidental injury.30 The precise role that prematurity plays
in unexplained fractures, whether it is a primary biological
role of bone disease of prematurity or a secondary social
association, is unclear and warrants further investigation.

It is difficult to recommend which children seen in the con-
text of an evaluation of fractures warrant further study to
determine if they have OI. Our studies failed to identify clear
clinical criteria that separated those with OI from those with-
out, perhaps because we did not enrol them in the 32-41, but
ourselves and did not have complete information. On the
other hand, none of the children who had other signs of abuse
was identified as having OI and this identifies a group for
whom additional study is not warranted. Finally, it is likely
that the combination of studies of protein production by cul-
tured fibroblasts and molecular genetic analysis of the type I
collagen genes will identify a small number of additional chil-
dren with OI. We are aware that complementary studies have
found abnormalities not found by the other method, although
the rate seems quite low.

Although the majority of infants we studied were assessed
by child protective services (CPS), we know little about how
these biochemical test results are used in the civil or criminal
justice system. Testing for OI presents an unusual situation in
the legal system in that testing one person for the presence of
a genetic condition may have a bearing on the health of a par-
tial and, conversely, on the ability of that parent to continue in
the household. Few other tests have such a role and it is
important that as genetic testing becomes more common, and
the use of test results in the civil and criminal justice system
becomes more frequent, we develop methods to ensure that
the results are used in both a scientifically valid fashion and
with compassion.

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