Bilirubin (serum, plasma)

1 Analyte

1.1 Name of analyte
Bilirubin

1.2 Alternative names
None

1.3 NLMC code

1.4 Description of analyte
Bilirubin is a linear tetrapyrrole (MW 585 Da), the final product of the breakdown of the cyclic tetrapyrrole ring of haemoglobin, myoglobin and the cytochromes. Bilirubin is insoluble in water. In plasma it is present in three forms:

- albumin-bound (reversibly: 'unconjugated bilirubin'), normally the major component
- conjugated
- delta bilirubin (conjugated bilirubin covalently bound to albumin).

Bilirubin is transported in the bloodstream to the liver bound to albumin; it is taken up by the liver and rendered water soluble by conjugation with glucuronic acid, principally to the diglucuronide, which is excreted in the bile. Small amounts are absorbed from the gut and re-excreted. For practical purposes, the presence of conjugated bilirubin in the plasma always indicates a pathological process. Delta bilirubin becomes present in significant quantities only in prolonged cholestasis.

Only conjugated bilirubin can be excreted in urine. Thus the detection of bilirubinuria also indicates a pathological process.

Note that the while the clearance of conjugated bilirubin from the plasma is rapid, that of delta-bilirubin depends on the clearance of albumin, and so is longer.

1.5 Function of analyte
Bilirubin is a waste product, destined for excretion. It has antioxidant properties but it is not known if this is of physiological significance.

2 Specimens and handling

2.1 Bilirubin is measured in serum or plasma, although its *detection* in urine (using dipsticks) is also informative (see 1.3). Non-invasive transcutaneous photometric measurement at point of care using a dedicated instrument 'bilirubinometer' is practised in infants to guide the need for blood to be drawn to guide treatment for neonatal jaundice.

2.2 Bilirubin is photosensitive, and specimens should be protected from light when highly accurate measurements are required. This is especially important with specimens collected from neonates.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

1. In the investigation of jaundice (yellow-orange discolouration of the skin and sclerae due to the presence of bilirubin)
2. As an index of hepatobiliary dysfunction. Bilirubin is typically measured as part of a panel of 'liver function' tests. The excess bilirubin in hepatobiliary disorders is typically totally or mainly *conjugated*.

3.2 Limitations

1. Serum [bilirubin] can be elevated to at least 50 µmol/L without jaundice being clinically apparent.
2. Neither hepatic nor biliary disease invariably causes an increase in serum [bilirubin].
3. An increase in serum [bilirubin] is not specific to hepatobiliary disease; it can occur in haemolytic conditions or conditions of ineffective erythropoiesis. The excess bilirubin in these instances is *unconjugated*.

4 Analytical considerations

4.1 Analytical methods

1. Diazo methods

These are based on reaction with diazotised sulfanilic acid (the diazo reagent) to produce two coloured azodipyrroles that can be measured spectrophotometrically, either at 530 nm or, after addition of alkaline tartrate, at 530 nm The reaction is accelerated by alcohol and a variety of other 'accelerators' (e.g. sodium benzoate) that cause the dissociation of unconjugated bilirubin from albumin. In the presence of an accelerant, both conjugated (including delta) and unconjugated bilirubin (together comprising total bilirubin) are measured; this is also termed 'indirect' bilirubin. In the absence of an accelerant, only the conjugated ('direct') bilirubin is measured. The difference is considered to be a measure of unconjugated bilirubin. It is important that no unconjugated bilirubin reacts in 'direct' methods: this can be avoided by maintaining a reaction pH of ~1.0.

2. High performance liquid chromatography (HPLC)

HPLC methods are capable of measuring the various bilirubin fractions present in plasma separately. This can be achieved using a Micronex RP-30 column but for practical purposes the use of direct and indirect diazo techniques provides sufficient clinical information for diagnostic purposes.

3. Enzymatic

These methods employ the enzyme bilirubin oxidase (EC1.3.3.5) to convert bilirubin to biliverdin. The reaction is followed by measuring the fall in absorbance at 425 or 460 nm. Separate quantitation of the different species of bilirubin is achieved by using different reaction pH conditions.

4. Spectophotometric

This method involves the measurement of absorbance at 437nm, the maximum absorbance of bilirubin. This is the basis of the method used in bilirubinometers.

4.2 Reference method

The reference method is based on the diazo method.

4.3 Reference materials

The reference material is human serum with added purified bilirubin (SRM 916a (National Institute of Standards and Technology, Gaithersburg, MD, USA)).

4.4 Interfering substances

The only major interfering substance is haemoglobin, but individual manufacturer's assays may suffer interference with certain drugs.

4.5 Sources of error

Exposure of specimens to light may cause photolysis of bilirubin and reduce its concentration.

5 Reference intervals and variance

5.1.1 Reference interval (adults): ≤25 μmol/L; conjugated 2 μmol/L

5.1.2 Reference intervals (others): ≤25 μmol/L (though ≤200 μmol/L may occur in the first 14 days of life (peak at 3-4 days)) as a result of 'physiological' jaundice (see 6.1 (4)).

5.1.3 Extent of variation

5.1.3.1 Interindividual CV: 35%

5.1.3.2 Intraindividual CV: 25%

5.1.3.3 Index of individuality: 0.71

5.1.3.4 CV of method: 13%

5.1.3.5 Critical difference: 80%

5.1.4 Sources of variation

In healthy individuals, the distribution of bilirubin concentrations is skewed to the right, reducing the precision of the upper reference limit. The high prevalence of Gilbert's syndrome (see 7.1(3)) is an additional problem.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation

1. Jaundice (adults)

The demonstration that an excess of bilirubin in the serum is conjugated (as may be inferred from the detection of bilirubinuria) is indicative of hepatobiliary disease but does not help distinguish between the possible causes. The transport of conjugated bilirubin from hepatocytes into the biliary system is an active process. Thus conjugated hyperbilirubinaemia can occur in:

- hepatocellular damage (hepatitis)
- intrahepatic cholestasis (e.g. cirrhosis)
- extrahepatic cholestasis (e.g. due to obstruction of the bile ducts by pancreatic carcinoma).

Purely unconjugated hyperbilirubinaemia is usually due to a haematological disorder causing increased red cell breakdown, but can occur as a result of impaired uptake by the liver of decreased conjugation.

2. Hepatobiliary function

Measurements of bilirubin can be used to monitor the course of hepatobiliary disease. For example, a fall in concentration following a procedure aimed at relieving obstruction suggests that this has been successful.

3. Haematological disorders

An elevated [unconjugated bilirubin] is characteristic of haemolytic conditions (e.g. congenital spherocytosis) and conditions in which there is ineffective erythropoiesis (e.g. vitamin B12 deficiency). In adults, [total bilirubin] very rarely exceeds 100 μmol/L in such conditions.

4. Neonatal jaundice

Considerations regarding jaundice in adults are also applicable to neonates, but additional factors apply:

- the neonatal liver is immature and haem turnover is increased. This can cause a (transient) increase in plasma [unconjugated bilirubin] and 'physiological' jaundice
- in conditions causing unconjugated hyperbilirubinaemia, plasma bilirubin can exceed 100 μmol/L. Concentrations in excess of 300 μmol/L carry a risk of causing brain damage (kernicterus)
- some causes of jaundice (e.g. the more severe inherited disorders of bilirubin metabolism) present uniquely or principally only in the neonatal period
- the precept that bilirubinuria indicates an excess of conjugated bilirubin in plasma applies in neonates as well as in adults and it should always be further investigated.

6.2 Confounding factors

Although hyperbilirubinaemia is characteristic of many conditions, it is diagnostic of none. Except when used to monitor the progress of a condition already diagnosed, the significance of hyperbilirubinaemia can only be assessed in relation to the results of other investigations (see 7.1.1)

7 Causes and investigation of abnormal results

7.1 High concentrations

7.1.1 Causes

Hyperbilirubinaemia has been classified in various ways, e.g. pre-hepatic, hepatic and post-hepatic. None is entirely satisfactory, as more than one mechanism may be operating in individual cases.

1. Increased production of bilirubin may exceed the ability of the liver to conjugate and excrete the pigment, causing an increase in the plasma [unconjugated bilirubin]. However, the capacity of the liver to process bilirubin exceeds the normal rate of production, so that increased production does not necessarily cause hyperbilirubinaemia. Specific causes include:

- haemolysis (autoimmune, congenital and other haemolytic anaemias)
- ineffective erythropoesis
- rhabdomyolysis (because of increased breakdown of myoglobin).

2. Decreased hepatic uptake can be caused by several drugs, e.g. rifampicin.

Although bilirubin uptake may be decreased in hepatocellular disease, the excess bilirubin in the plasma is primarily conjugated, reflecting decreased secretion into the biliary system.

3. The conjugation of bilirubin may also be impaired in hepatocellular disease, but any excess bilirubin in the plasma is primarily conjugated.

Two inherited conditions of impaired of bilirubin conjugation can cause unconjugated hyperbilirubinaemia. These are:

- Gilbert's syndrome (common and benign) typically causing only mild hyperbilirubinaemia (≤100 µmol/L) and sporadic jaundice, typically in young adults
- Crigler-Najjar syndromes types I and II, causing severe hyperbilirubinaemia and typically presenting in infancy.

4. Impaired secretion of bilirubin into the biliary system can occur in hepatocellular diseases. It can also be caused by drugs, including chlorpromazine, carbamazepine and erythromycin. Impaired secretion bilirubin into the biliary system is a feature of two rare inherited disorders, Rotor syndrome and Dubin-Johnson syndrome.

5. Biliary obstruction can cause diffusion of conjugated bilirubin from the biliary system into the bloodstream. Such obstruction can be intrahepatic (e.g. cirrhosis) or extrahepatic (e.g. sclerosing cholangitis, carcinoma of the head of the pancrease, causing obstruction through external pressure on the common bile duct.

7.1.2 Investigation

Bilirubin is usually measured as part of a panel of 'liver function' tests, including albumin, total protein, a transaminase (aminotransferase) and alkaline phosphatase. Appropriate investigation will depend on the results of these tests.

1. Results of other 'liver function' tests are normal

Values up to 20% higher than the upper limit of normal (ULN) may be statistical outliers.

With values ≤1.5 x ULN, the urine should be tested for bilirubin. If the result is negative, it can be inferred that the excess bilirubin is unconjugated and the measurement should be repeated within three months unless there is clinical suspicion of disease.

If a value of ≤1.5 x ULN is confirmed or an initial measurement is >1.5 but ≤3 x ULN, the possibility of a haematological cause should be investigated by performing a full blood count (including reticulocytes), examining a peripheral blood film and performing a Coombs' test. If haemolysis is excluded, the most likely diagnosis is Gilbert's syndrome. If required, this can be confirmed by a genetic test.

Values >3 x ULN should be investigated further regardless of whether other 'liver function tests' are abnormal. If the conjugated fraction is >50% of the total, ultrasound imaging of the hepatobiliary system is indicated; if the unconjugated fraction is >70% of the total, investigations for haemolysis should be performed.

2. Results of other 'liver function tests' are abnormal

The investigation of hyperbilirubinaemia with abnormal liver function tests is likely to be informed by any accompanying clinical abnormalities. However, predominant elevation in serum aminotransferase (transaminase) activities in comparison with serum alkaline phosphatise activity suggests hepatocellular dysfunction. This should be investigated by viral and autoimmune serology. A predominant increase in alkaline phosphatase suggests biliary obstruction (either intra- or extrahepatic) and should be investigated initially by ultrasound examination, further investigation depending on the findings.

7.2 Low concentrations

7.2.1 Causes

The lower limit of the reference range for bilirubin is zero. Pathologically low values do not occur.

7.2.1 Investigation

Not applicable

7.3 Note

Jaundice is common in neonates. If it develops more than 24 h after birth, lasts not more than 14 days and is due only to unconjugated bilirubin, it may be benign 'physiological' jaundice. A high [bilirubin] (>300 µmol/L) carries a risk of brain damage and requires intervention. Conjugated hyperbilirubinaemia is always pathological, as is hyperbilirubinaemia presenting in the first 24 h or persisting after 14 days.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

Sensitivity of serum bilirubin levels in selecting patients for magnetic resonance cholangiopancreatography. Haroun AA, Al-Hadidi AM, Tarawaneh IS *et al.* Hepatogastroenterology 2007;54:995-998. *Increased [conjugated bilirubin] reported to have 77% sensitivity and 80% specificity for predicting abnormal findings on MRCP.*

9 Systematic reviews and guidelines

9.1 Systematic reviews

All systematic reviews identified relate to neonatal jaundice and the efficacy of procedures for predicting possible bilirubin encephalopathy (kernicterus); the consensus appears to be that a combination of early measurement of serum total bilirubin with assessment of other risk factors cannot reliably predict those infants at high risk of the condition. E.g. Trikalinos TA, Chung M, Lau J, Ip S. Systematic review of screening for bilirubin encephalopathy in neonates. Pediatrics. 2009;124:1162-1171.

9.2 Guidelines

NICE Clinical Guideline 98 (2010) Neonatal Jaundice. http://publications.nice.org.uk/neonatal-jaundice-cg98 (accessed 25.iv.2012)

C*ontains comprehensive guidance on the investigation and management of jaundice in infants.*

No guidelines identified in relation to jaundice in adults.

9.3 Recommendations

Recommendations identified complement the advice provided in guidelines with regard to jaundice in infants. No specific recommendations identified in relation to jaundice in adults. Recommendations given for the investigation of isolated increases in serum [bilirubin] are based on expert opinion, not clinical evidence.

10. Links

10.1 Related analytes

None

10.2 Related tests

Measurement of other indices of liver function, including the standard 'liver function' tests (see 7.1.1), is often valuable in the interpretation of an abnormal [bilirubin]. In general, however, laboratory tests on their own rarely provide a final diagnosis: their results tend to indicate types of pathological process.

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