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Short reports

Whole blood assay of theophylline concentrations using immunochromatographic stick at the bedside

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SUMMARY Blood was collected from 77 patients for estimation of theophylline concentration by whole blood assay using an immunochromatographic stick (Acculevel, Syva UK, Maidenhead, Berkshire). Results were validated by high performance liquid chromatography (HPLC). The stick method was rapid, reliable, required no technical expertise, and produced results equivalent to those obtained from assay from HPLC.

Methylxanthines are commonly used in the treatment of asthma. The necessity for monitoring theophylline concentrations in serum has been emphasised by many authors, who have reported wide variations in the pharmacokinetics of theophylline not only among patients but also within the same patient.[1 2] Theophylline concentrations measured in saliva have been shown to be unreliable.[3]

Various techniques are used to measure theophylline concentrations in serum. The purpose of this study was to compare results obtained by high performance liquid chromatography (HPLC) with those of a simple stick assay carried out at the bedside.

Patients and methods

Seventy seven patients, aged from 1 to 14 years, were studied at two hospitals. They all received theophylline orally or aminophylline intravenously. Informed consent was obtained from the parents.

A sample of 1·0 ml of whole blood was obtained by venepuncture, and 12 µl were aspirated into a graduated pipette. The serum was separated from the remaining sample and assayed by HPLC. The stick assay was performed by adding 12 µl of whole blood from the graduated micropipette to reagent 1, which contained 1 ml conjugate of glucose oxidase, horseradish peroxidase labelled theophylline, and sheep antiserum in 0·1 M phosphate buffered saline at pH 7·0. The cassette contained chromatography paper impregnated with mouse monoclonal antibodies to theophylline. The patient's theophylline concentration and enzyme labelled theophylline rose together by capillary action, binding to the immobilised antibody sites on the cassette chromatography paper, with glucose oxidase saturating the remaining cassette paper by eight minutes. The cassette was then placed in reagent 2 containing 10 ml of 4-chloro-1-naphthol and glucose in 0·01 M phosphate buffered saline at pH 6·5. The glucose oxidase on the strip converted glucose and oxygen to gluconate and hydrogen peroxide which, in the presence of peroxidase and 4-chloro-1-naphthol, produced an insoluble blue precipitate; this formed a colour bar on the chromatography strip in five minutes. The height of the bar in mm was then read, and the level of the theophylline determined from the concentration chart appropriate for the batch of Acculevel sticks used.

HPLC was performed at the Queen Elizabeth Children's Hospital by extracting the methylxanthines together with an internal standard (3-ethylxanthine) from plasma, using a specially developed solid phase sample preparation tube and organic solvents, which were dried under oxygen free nitrogen at <40°C. The extracts were reconstituted with HPLC mobile phase, and the methylxanthines were separated on a 25 cm, 3 µ Apex 1 octadecyl-silane (ODS) column with a solvent gradient, and detected at 273 nm.[4]

Reverse phase HPLC was performed at Paddington Green Children's Hospital by the following method: isocratic chromatographic separation was carried out using an ODS 3 Partisil 10 µ column. The internal standard 8-chlorotheophylline was added to 200 µl of patient's serum, and 440 µl of acetonitrile. This was spun for 30 seconds, centrifuged for 10 minutes, and the supernatant evaporated completely under vacuum. The residue was reconstituted with 200 µl of half strength mobile phase, and after

spinning a 40 µl sample was injected on to the liquid chromatography column, and detected at 273 nm.

Differences between methods were analysed using a paired *t* test.

Results

The mean difference between the theophylline concentrations obtained from the alternative assays of the individual patient samples was 0·38 mg/l. There was no significant difference between the two assay methods ($t=1·65$).

The coefficient of variation of repeated measurements for the Acculevel stick at 9 mg/l was 5·36%.

Discussion

There are various ways of measuring methylxanthines, including reverse phase HPLC,[4] gas liquid chromatography, and high pressure cation exchange chromatography, but these methods require considerable expertise. Homogenous enzyme immunoassay is simpler but still requires specific machinery which must be carefully used to obtain reliable results.[5] Theophylline stick assays are simple, reliable, and can be used by clinicians after limited training. An alternative theophylline stick assay requires the separation of plasma and a specific machine for measurement.[6]

This whole blood theophylline stick assay requires neither separation of plasma, nor expensive technology. It is particularly suitable for use outside normal working hours, casualty departments, and in small hospitals that do not have access to a therapeutic drug monitoring service. It is highly acceptable, particularly to children, as it requires only 12 µl of whole blood obtained by finger prick. As with all stick assays, care must be taken to follow the correct procedure, or inaccuracies will result.

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Monitoring of end tidal CO_2 in neonatal intensive care

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SUMMARY The use of monitoring end tidal carbon dioxide pressure ($PetCO_2$) in neonatal intensive care was studied in 19 infants with respiratory disease. $PetCO_2$ correlated poorly with arterial pCO_2, the relation being principally determined by the severity of pulmonary disease. Monitoring end tidal CO_2 cannot be recommended for neonates with pulmonary disease.

In modern neonatal intensive care units the continuous monitoring of arterial carbon dioxide partial pressure ($PaCO_2$) has become increasingly important. End tidal pCO_2 monitors, suitable for use with neonates, have recently been developed and have been proposed as alternatives to transcutaneous pCO_2 monitors for non-invasive monitoring of CO_2 content in ill premature infants. We therefore examined the accuracy and clinical usefulness of measuring the end tidal partial pressure of CO_2 ($PetCO_2$) in such a group of infants.

Method

The monitor used was an Engstroem Eliza infrared capnometer. This was connected to the patient's respiratory circuit by a 15 mm endotracheal tube