Science Digest

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Ketoacidosis at autopsy

Metabolic acidosis is a common finding resulting from diabetes, starvation, and alcohol abuse with an increase in b-oxidative ketone bodies, (primarily β-hydroxybutyrate). In causes of unexplained deaths, elevated ketone bodies may be detected in the blood, vitreous fluid or other body fluids e.g., CSF. A recent systematic review on ketoacidosis at autopsy from the UK indicated that ketoacidosis may be an important factor in unexplained deaths (1). The authors cite a USA study indicating that that of the 93 deaths due to ketoacidosis in Baltimore, USA over a six-year period, 32 had no known cause of diabetes and they overview the cause of ketoacidosis. During the UK study period, 816 cases, 97 of these had β-hydroxybutyrate performed. Where vitreous fluid was unavailable, raised blood acetone was an indicator for ketoacidosis. A raised vitreous β-hydroxybutyrate of >960mmol/L was taken as being associated with significant mortality and was used to select cases. All data was extracted from autopsy reports and medical histories, and included route biochemistry profiles, vitreous fluid chemistry and blood and vitreous fluid ethanol. In total 42 patients clinical, pathological and biochemical findings were obtained. Of the 42 patients over a two-year period, 50% died of alcoholic ketoacidosis, 19% of diabetic ketoacidosis and 12% of both.

The authors also indicate that the differing concentrations of glucose and β-hydroxybutyrate can be obtained at differing sample sites at autopsy. It was emphasised that other causes of ketoacidosis may contribute to death such as starvation, infection, trauma and hypothermia, and that ketoacidosis should be excluded when there is no clear cause of death.

Paracetamol and sperm function

In recent years there have been numerous publications relating to decreasing sperm counts leading to increasing concern of male reproductive health. To-date no single cause for the decreased sperm counts has been identified although some evidence points to environmental endocrine disruptors. These environmental chemicals have been shown to disrupt the CatSper [Ca2+] channel which regulates important aspects of sperm function including motility. Previous research has indicated that men with high urinary levels of paracetamol (acetaminophen) had impaired sperm motility, increased sperm fragmentation and an increased time-to-pregnancy. In the present research from Denmark, the authors investigated the effect of paracetamol and its active metabolites on sperm function both in-vivo and in-vitro (2). Using healthy male volunteers, blood, semen and urine samples were collected prior to and during when paracetamol and its metabolites were administered. Following preparation, the samples were analysed LC/MS, UHHPLC-ESI-HRMS for metabolite identification as well as changes in [Ca2+] and fatty acid amide hydrolase activity (FAAH). All participants demonstrated significantly increased paracetamol exposure by day 3 mirroring concentrations in all three samples. One metabolite (N-arachidonoyl phenolamine, AM404) demonstrated a dose dependent increase in sperm intracellular calcium via activation of the CatSper channel. This metabolite can be conjugated to arachidonic acid by FAAH to form N-arachidoyl phenolamine which also induced calcium influx in sperm. Sperm motility was also decreased via CatSper.

The authors conclude that paracetamol and its metabolites are transferred into seminal fluid have a direct effect on CatSper-mediated calcium influx and that sperm themselves can metabolise paracetamol metabolites which will interfere with sperm [Ca2+] signalling. This is turn will influence sperm motility and delays in achieving a pregnancy.

Stability of SARS-COV-2 nasopharyngeal swabs

Collection, storage and stability of swabs for viral investigations is an important aspect for detection and monitoring of infections. Setting aside the pre-analytical requirements for the swabs, knowing that swabs for viral analysis can provide a reliable result if they have to be transported and possibly stored prior to analysis is vital for diagnosis. A short report from the USA has investigated the storage and stability of nasopharyngeal swabs for the detection of SARS-COV-2 using three different RT-PCR platforms (3). The authors discuss previous work which provided evidence for viral investigation swabs for a range of viral related disorders such as influenza, enterovirus, adenovirus and herpes simplex virus, indicating their stability for viral detection by RT-PCR for up to seven days. In the present work, the authors pooled 30 remnant nasopharyngeal swabs from known SARS-COV-2 positive patients and aliquoted them into 126 samples. These were split and stored for 21 days at both 18 to 25°C and 2 to 8°C and sampled at daily over 21 days. Three different automated RT-PCR platforms were used, Luminex ARIES, Panther Fusion and Abbott m2000. The 'stability samples' were analysed along with the routine clinical samples and the single cycle threshold was recorded, providing 244 data points. In addition, the authors tested seven SARS-COV-2 patient samples that had been stored at 4°C for 35 days. The qualitative detection of the virus was 100% for all three systems and storage at the two temperatures demonstrated little difference over time. All three instrument platforms performed extremely well and the seven patient samples tested positive after 35 days at 4°C. The authors concluded that the storage of nasopharyngeal swabs for SARS-CoV-2 detection could be reliably used for the detection of SARS-COV-2 infections.

Cell-free RNA and pregnancy outcome

Although in the Western world pregnancy is considered comparatively safe, there are still unexpected complications which may develop during the pregnancy. Typically, gestational diabetes and hypertension of pregnancy still can remain serious issues in pregnancy management. Of the two, hypertensions in pregnancy and pre-eclampsia still remain life-threatening disorders to both the woman and the fetus, with 14% of maternal deaths being related to these disorders, only below maternal haemorrhage. Hypertension and pre-eclampsia typically develop after 20 weeks' gestation, although pre-eclampsia may develop at any stage of pregnancy. Two recent publications have investigated the use of maternal blood cell-free RNA (cfRNA) to predict the outcomes of pregnancy (4,5). The first (4), a multicentre international collaboration, used transcriptome data analysis from 1,840 racially diverse pregnancies, retrospective analysis of 2,539 banked pregnancy plasma samples and a range of gestational ages. The presence of cfRNA in the maternal blood correlated with ultrasound gestational age assessment. The authors demonstrated that the maternal plasma and placental cfRNA correlated with fetal gene sets. Of these three genes identified the probability of pre-eclampsia with a positive predictive value of 32.3% with maternal BMI or race having no effect. The second publication from the USA (5) published a week later, identified a panel of 18 genes that indicate pre-eclampsia pathology at between 5 to 18 weeks of gestation using 404 plasma samples from 199 pregnancies associated with pre-eclampsia. Both sets of authors conclude that the samples and data indicate evidence of early abnormal placentation and endothelial dysfunction and that non-invasive (to the fetus) monitoring cfRNA could indicate the advent of pre-eclampsia and the signature for organ dysfunction. In conclusion they propose that clinical validation would be required to determine the pathogenesis of pre-eclampsia.

Point of care glucose meter standardisation

Point of Care (POCT) is rapidly becoming the 'norm' for a number of analytes such as glucose. The POCT may be performed in a number of settings such as an individual's home, pharmacies and various POCT stations around a hospital. As new diagnostic technologies emerge, POCT is estimated to grow by 12 to 16% per year. Despite the overall reliability of the instruments themselves, issues arise with the individual's use of the testing system, quality control, result flags, maintenance etc. In a recent publication from the USA a multi-disciplinary group investigated POCT glucose standardisation over a single year (6). This involved approximately 2.4million POCT glucose assays undertaken by 17,000 operators using more than 700 meters. All glucose meters in the survey were anonymised depending on the manufacturer and their distribution and use was spread over all potential users in a hospital setting. The performance of four glucose meters was assessed and validated in a single certified laboratory using the same instruments. A 16-point spreadsheet of operational criteria using a Likert scoring scale was used to compare the performance of each selected instrument. Blood samples included capillary, arterial and venous samples. The validated reference method was the hexokinase colorimetric method on either ABL or Roche c502 analysers. Overall, the analytical performance of all the meters was comparable for both laboratory and clinical evaluation. One meter however, ranked the highest for usability, implementation and streamlined interface connectivity. The authors conclude that standardisation was an integrated system-wide organisational structure requiring a centralised POCT oversight. They also commented that certain instruments were not validated for use with both the critically ill or neonatal venous blood samples. In addition, they commented on the poor connectivity of some system with LIS including patient identification and operators interrupting power on/off sync-sequences. From this work the authors are now investigating the standardisation of other diagnostic based analyser systems. **NOTE:** The authors did not identify the meters in the publication.

Are all SARS-COV-2 methods created equal?

The advent of the world-wide SARS-COV-2 outbreak and declaration of a pandemic by the WHO on March 2020 required the rapid development of an accurate test both for diagnosis and epidemiological tracking of the infection. The identification of the virus sequence resulted in the development and publication of PCR assays, subsequently resulting in establishment of numerous commercial and laboratory-based SARS-COV-2 tests and procedures. Subsequent to the initial outbreak, molecular testing identified a number of SARS-COV-2 variants, which raised the question whether all the PCR diagnostic tests were sufficiently reliable to detect SARS-COV-2 variants. A recent international survey performed on behalf of the International Federation of Clinical Chemistry and Laboratory Medicine (Molecular Diagnostics Committee) investigated the global overview of test methods, laboratory procedures and quality assessment of methods used to detect SARS-COV-2 (7). Using an anonymised online survey, they addressed laboratory demographics, techniques, and virus detection and variant sequencing. A total of 273 laboratories from 49 countries were surveyed. Of these 92.2% used RT-PCR testing, however, the majority of these did not test for SARS-COV-2 variants. In addition, 33.2% of laboratories did not participate in an external quality assurance programme. The testing numbers varied considerably with 17.8% of laboratories performing 0 to 100 tests/week and the majority (31.1%) performing 100-1770 tests/week; 26.6% of laboratories were performing 1400 to 7000 tests/week. Although the majority of participants used RT-PCR there was wide variability on the actual methods used. Reporting was as cycle threshold in 51.1% and Positive/Negative in 57.7% of positives. 4.5% reported copies/mL. For the variants of concern, 25% of laboratories genotyped positive cases in their own laboratory, the remainder did not and 51.9% did not have their positive samples sequenced at all. As a result of the survey the Committee identified the lack of standardisation across different laboratories and countries, as well as sample collection processing and result reporting and concluded that there was insufficient quality assurance being performed by diagnostic testing laboratories for SARS-COV-2 testing. They also commented on comparability of antigen-based methods and molecular methods in the context of analytical and clinical performance including issues relating to quality assurance.

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