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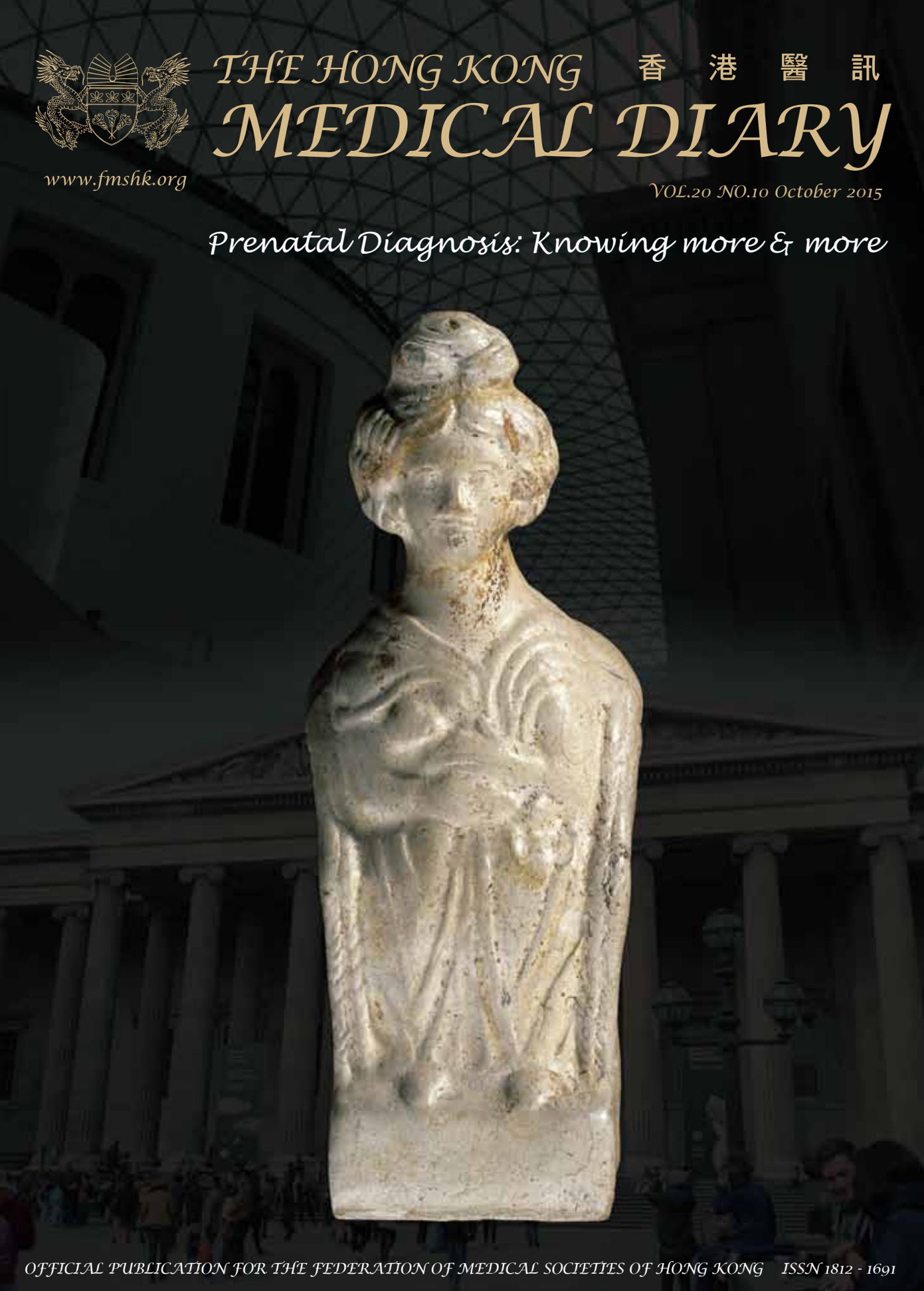
Prenatal Diagnosis: Knowing more & more



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Contents

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The Cover Shot

Mother and baby
From: Welwyn, Hertfordshire
Date: 2nd century AD

This little statuette shows a mother-goddess sitting in a chair breastfeeding her baby. Roman medical books recommended that children should be breastfed either by their mother or a wet-nurse until they were 18 months to 2 years old. Roman wisdom two thousand years ago still holds true in our days and times. (© Trustees of the British Museum)

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New Algorithms in Prenatal Diagnosis

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Editor

Dr Wing-cheong LEUNG

Advances in prenatal molecular diagnostics have revolutionizsed our traditional approach in prenatal diagnosis. New algorithms in prenatal diagnosis are evolving. (Figure 1) By knowing more & more with these new algorithms, are we moving towards Eugenics in Prenatal Diagnosis? To a certain extent we are! On the other hand, the ultimate goal is to provide enough information for pregnant women & their families to make choices for their next generations.

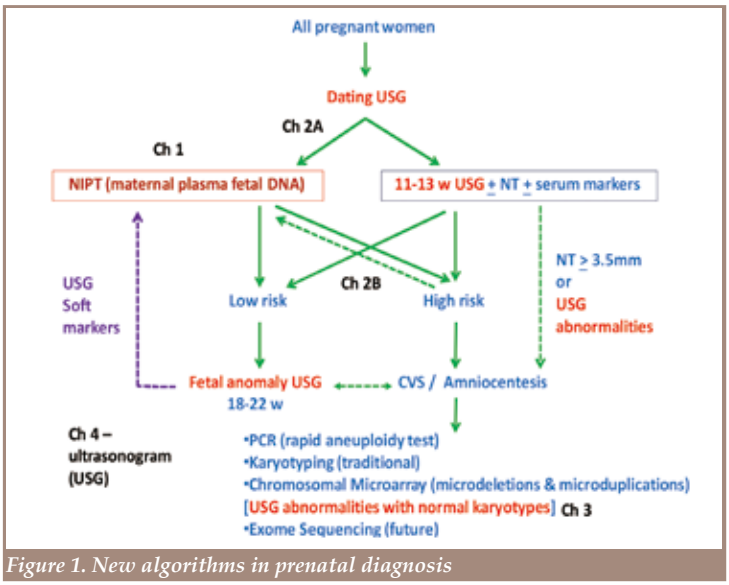


Figure 1. New algorithms in prenatal diagnosis

Screening at 11-13 weeks by a combination of maternal age, foetal nuchal translucency thickness (NT) measurement by ultrasound, and maternal serum free beta-hCG & PAPP-A, can identify 90% of foetuses with trisomy 21 (Down syndrome) and other major aneuploidies (such as trisomy 18 and trisomy 13) for a false-positive rate of 5%. The performance is highly reproducible worldwide which has also been demonstrated in our Hospital Authority universal Down syndrome screening programme starting from 2010. Additional ultrasound and maternal serum markers with different contingent policies have been studied to further improve the detection rate and reduce the false-positive rate. But the most important recent development must be the non-invasive prenatal testing (NIPT) for foetal chromosomal abnormalities using maternal plasma cell-free foetal DNA discovered by Prof Dennis LO from Hong Kong. The detection rate for Down syndrome using NIPT is more than 99% with a false-positive rate as low as 0.1%. NIPT can be performed using maternal blood sample from 10 weeks onwards. NIPT is currently available for secondary screening for pregnancies with positive conventional Down screening as well as for primary screening for Down syndrome. We are most honoured to have Prof Rossa CHIU, an international renowned expert in NIPT, to be the author of *Chapter 1* (CME article): NIPT – a breakthrough in prenatal diagnosis.

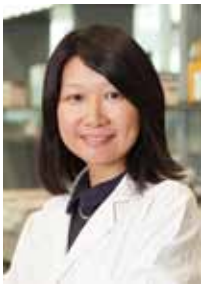
This was a hot debate in our College (HKCOG) Postgraduate Study Day (9/11/2014) on whether NIPT should be used as primary screening for foetal Down syndrome. Dr TK LO and Dr LW LAW kindly agreed to continue their debate by writing for (*Chapter 2A*) and against (*Chapter 2B*) this notion respectively.

In the 21st century, traditional karyotyping is no longer adequate for prenatal diagnosis. There are advances in prenatal molecular diagnostics including PCR (polymerase chain reaction) as a rapid aneuploidy test, chromosomal microarray / array CGH (comparative genomic hybridisation) to detect microdeletions & microduplications, and the future exome sequencing (sequencing all the protein-coding genes in a genome). In *Chapter 3*, Dr Anita KAN & Dr Kelvin CHAN will discuss the role of chromosomal microarray.

Ultrasound examinations (11-13 weeks for NT + foetal structural abnormalities; 18-22 weeks for foetal structural abnormalities) remain to play a pivotal role in these new algorithms, which is making prenatal diagnosis more effective and comprehensive. Dr WK YUNG & Dr WL LAU will explain the essential role of ultrasound in *Chapter 4*.

I am indebted to all the authors / friends for their time & effort out of their busy schedules in preparing the manuscripts. But I am sure readers of the Hong Kong Medical Diary will find this Medical Bulletin informative and interesting.

Chapter 1
Non-invasive prenatal testing – a breakthrough in prenatal diagnosis



Prof Rossa WK CHIU

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This article has been selected by the Editorial Board of the Hong Kong Medical Diary for participants in the CME programme of the Medical Council of Hong Kong (MCHK) to complete the following self-assessment questions in order to be awarded 1 CME credit under the programme upon returning the completed answer sheet to the Federation Secretariat on or before 31 October 2015.

Screening or testing for foetal diseases and conditions is an important part of prenatal care. To perform prenatal diagnosis of foetal genetic or chromosomal diseases, genetic material of the unborn child would need to be obtained. Conventionally, such foetal genetic material is sampled via invasive procedures such as chorionic villus sampling or amniocentesis. Those procedures are associated with a small but not immaterial risk of foetal miscarriage. Therefore, it has been the practice for many years to use non-invasive means, such as foetal ultrasonography and maternal serum biochemistry testing, to identify women whose pregnancy is deemed to be at high risk for a genetic or chromosomal condition. The high risk women would then be offered the option of invasive prenatal testing. However, those screening tests, such as the first trimester combined tests for Down syndrome detection, typically identify about 3% to 5% of women as high risk. On average, Down syndrome affects 1 in 700 pregnancies. Hence, the majority of the women labelled as "high risk" in fact do not carry a Down syndrome foetus and unnecessarily need to face the difficult decision of whether or not to undergo invasive testing. Consequently, it would be ideal if foetal DNA could be obtained safely with no harm to the foetus for genetic analysis. Here we describe that this option of non-invasive foetal DNA testing is now practically feasible and is a clinical reality.

Cell free foetal DNA in maternal blood

In 1997, Dennis Lo and colleagues reported the observation of chromosome Y DNA in the plasma and serum of women pregnant with male foetuses but not in women pregnant with female foetuses[1]. Because the chromosome Y molecules must have originated from the DNA of the male foetus and not the mother, this was the first demonstration of the presence of cell-free foetal DNA molecules in maternal circulation. Today, we understand that these DNA molecules originate from dying cells of the placenta, due to both normal cell turnover and pathologies. Being cell degradation products, these DNA molecules are not bound by a cellular membrane and are fragmented into short molecules. They are therefore referred to as circulating cell-free foetal DNA. In fact, all human subjects have cell-free DNA in their circulation. The great majority is from haematological cells. Hence, in maternal plasma, the foetal DNA circulates among a background of DNA from the mother's blood cells. Foetal DNA typically accounts for about 10% to 15% of the total plasma DNA[2]. The existence of cell-free foetal DNA in maternal plasma provided the means to develop DNA-based non-invasive prenatal tests (NIPT).

Down syndrome screening by cell-free foetal DNA testing

Down syndrome, typically caused by trisomy 21, is one of the commonest reason for a couple to seek prenatal screening. When a woman is pregnant with a Down syndrome foetus, the foetus would release extra amounts of chromosome 21 DNA into the plasma of the woman when compared with that of women pregnant with non-affected foetuses. Our group developed tests that aimed at detecting the increased chromosome 21 DNA content in maternal plasma[3,4]. Subsequently, clinical trials demonstrated that the test could detect 99% of Down syndrome foetuses with 0.1% false positive rate[2,5]. To achieve this level of accuracy, a sophisticated method, namely massively parallel sequencing, was needed to analyse at least tens of millions of DNA fragments in each maternal plasma sample. Besides its high accuracy, this test could be applied from early pregnancy, say 10 to 11 gestational weeks, and onwards. It is equally applicable during the rest of the gestational period, not limited to any gestational window.

However, due to the sophisticated instrumentation, NIPT is relatively expensive at present, costing several thousand Hong Kong dollars per case. Furthermore, due to the associated false-positive rate, though small, positive maternal plasma DNA test results still require definitive confirmation by invasive testing[6]. A 0.1% false-positive rate is still substantially lower than the 3% to 5% of false-positivity by maternal serum biochemistry screening. Therefore, a number of professional groups including the American College of Obstetricians and Gynecologists[6] recommended the use of the non-invasive maternal plasma DNA sequencing test for Down syndrome as a second-tier screening test or triage tool for pregnancies identified to be at high risk for Down syndrome by other tests or means. Recent publications suggested that the maternal plasma DNA sequencing test showed similar performance for both high and low risk pregnancies[7,8]. Thus, professional groups began to discuss whether the test could be applied to women of all risk groups[9,10]. Nonetheless, the test became available for clinical use in 2011 and

has now been established in over 100 countries. Its use has resulted in significant reductions in the number of amniocenteses performed worldwide[9].

NIPT of other chromosomal aneuploidies

Similar test principles could be applied for the non-invasive detection of trisomy 18 and trisomy 13. Indeed, detection rates of >90% and false-positive rates of <1% could be achieved[11]. Maternal plasma DNA sequencing has also been applied to the non-invasive detection for sex chromosome aneuploidies. Scientifically, it has been shown to be feasible to detect subchromosomal microdeletions and microduplications if many more plasma DNA molecules are analysed to obtain a higher resolution read-out of the maternal plasma sample[12]. In fact, detailed analysis of the chromosome dosage across the genome, namely a molecular karyotype, is scientifically achievable if hundreds of millions of maternal plasma DNA fragments are analysed[12,13]. At present, such a protocol is too costly to be implemented in a routine fashion.

Tips for test interpretation

False-negative results

The maternal plasma DNA sequencing test demonstrates high sensitivity and specificity for the detection of whole chromosome aneuploidies. But infrequently, there are false-negative and false-positive results. Investigations that have been conducted on the occasional false-negative cases so far revealed several likely causes for such results. First, it is most important that the foetal DNA amount in the sample, also termed the foetal DNA fraction, is adequate for the analysis[14]. Say for illustration purposes, when a maternal plasma sample contains no foetal DNA, the sequencing analysis would then be performed on millions of cell-free DNA molecules from the mother's blood cells and produce a report that is not informative for the foetus at all. This situation could be avoided if the testing laboratory includes the measurement of the foetal DNA fraction as a quality control parameter. Specimens that contain inadequate foetal DNA, namely below a threshold amount, would be flagged and typically a resubmission of another blood sample would be recommended.

In addition, the detection of mosaic chromosomal aneuploidies are more challenging. Mosaicism refers to the state when only a fraction of the foetal cells are affected. This means that the *effective* amount of foetal DNA from the affected chromosome might become inadequate for detection[14]. Let's consider a hypothetical example where a woman is pregnant with a male foetus with mosaic trisomy 18 where only 20% of the foetal cells show trisomy 18. When NIPT is conducted on the maternal plasma sample, the analysis shows that the sample contains 10% male DNA. Thus, the foetal DNA fraction is considered to be 10% and the specimen has passed the quality control requirement for report issuance. However, the trisomy 18 only affects one-fifth (20%) of the foetal cells. Therefore, the effective concentration of the trisomic cells in the sample is only 2% (one-fifth of the 10% foetal DNA in maternal plasma) and may be too small to be detected by the protocol. Mosaicism therefore lends to the potential for false-negative detection[14].

Another situation where false-negative results have been reported relates to discordances between the placental and foetus proper. There have now been a number of cases reported in the literature where the foetus is confirmed, such as by amniocentesis, to be affected by chromosomal aneuploidy while the NIPT results were negative due to low level mosaicism in the placenta[14,15]. The "foetal" DNA in maternal plasma in fact originates from the placenta. Therefore, when the abnormality is present at low concentration in the placenta, the effective amount of the DNA molecules from the affected chromosome would be too low for detection.

Yet another challenging scenario is when the size of the chromosomal aneuploidy is too small. For example, for the detection of subchromosomal aneuploidies, such as microdeletions or microduplications, the affected region that is contributing placental DNA for detection in maternal plasma is small. This is equivalent to the above-described situations when the effective amount of abnormal DNA is so small that the protocol might fail to detect it. Therefore, for tests that intend to address such subchromosomal aneuploidies, the protocol entails the analysis of much larger number of total DNA molecules from the sample to enhance the chance of detecting the finer abnormalities and hence, adds costs to the test[16]. In addition, the incidence of subchromosomal aneuploidies is much lower than trisomy 21. Therefore, even if both protocols have the same specificity, it is much more likely for a reported subchromosomal aneuploidy to be false-positive than for trisomy 21[9].

False-positive results

False-positive results could be a consequence of confined placental mosaicism[17]. Confined placental mosaicism refers to mosaic chromosomal abnormalities that are found in the placental cells but not the foetus proper. In fact, mosaic cytogenetic abnormalities are not uncommon in the placenta and have been reported in 2% of chorionic villus samples[18]. Malvestiti et al reported that only 13% of the mosaic chorionic villus abnormalities are also detected in amniocytes and thus are truly present in the foetus[18]. Because NIPT based on maternal plasma DNA analysis assesses circulating DNA that is of placental origin, in theory, NIPT would detect many of those mosaic abnormalities confined to the placenta that are not present in the foetus.

Another source of "false-positive" result is subclinical cytogenetic abnormalities of the mother. It is now appreciated that mosaic sex chromosome aneuploidies are not uncommon in the population. When NIPT analysis is performed on a maternal plasma sample, most of the analysed DNA in fact comes from blood cells of the pregnant women. If the woman is mosaic for 45, X0 or 47, XXX, the test may report these findings that may be misinterpreted as affecting the foetus. Wang et al observed that 8.6% of the sex chromosome aneuploidies reported by NIPT involved cases where additional investigations revealed the same findings among the maternal cells[19]. Based on these observations, Wang et al advocated the testing of maternal blood cell DNA and these additional findings should be taken

in consideration when one counsels the patients about the NIPT results[19]. However, knowing the presence of the maternal DNA findings does not provide analytical information to distinguish whether the foetus does or does not have the same chromosomal condition.

Incidental findings

Abnormalities detectable among circulating cell-free DNA are not limited to the prenatal setting. Cancer and some autoimmune diseases, like systemic lupus erythematosus, are also associated with abnormal plasma DNA profiles[20,21]. Therefore, it has happened where DNA abnormalities detected by NIPT in fact revealed the presence of maternal disease, such as cancer[22].

Future directions

Hong Kong has been at the forefront in terms of the development and adoption of NIPT. Since the clinical availability of NIPT for foetal chromosomal aneuploidies, the practice of prenatal screening has changed remarkably in Hong Kong and the rest of the world. Both clinicians and patients adapted to the availability of the test rapidly. Guidelines and recommendations from professional groups had been updated in real time as new clinical and scientific evidence merged. In fact, methods have been developed that allowed the non-invasive prenatal detection of foetal genetic diseases such as beta-thalassaemia, haemophilia and congenital adrenal hyperplasia[23-25], or even the entire foetal genome[23]. It is expected that when the costs of the technology reduces, the newer protocols will one day be introduced into clinical use. Lastly, the emergence of NIPT and the rapid surge in its clinical adoption rate might signal the advent of the era of the practice of genomic medicine across other branches of medicine.

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MCHK CME Programme Self-assessment Questions

Please read the article entitled "Chapter 1 - Non-invasive prenatal testing – a breakthrough in prenatal diagnosis" by Prof Rossa WK CHIU and complete the following self-assessment questions. Participants in the MCHK CME Programme will be awarded CME credit under the Programme for returning completed answer sheets via fax (2865 0345) or by mail to the Federation Secretariat on or before 31 October 2015. Answers to questions will be provided in the next issue of The Hong Kong Medical Diary.

Questions 1-10: Please answer T (true) or F (false)

1. For Down syndrome screening, non-invasive prenatal testing by cell-free DNA analysis in maternal plasma has lower false-positive rates than the conventional maternal serum biochemistry tests.
2. The cell-free foetal DNA molecules that circulate in maternal plasma come mainly from the placenta.
3. DNA-based non-invasive prenatal tests have high positive predictive values and therefore confirmatory testing by invasive sampling of foetal cells is unnecessary.
4. DNA-based non-invasive prenatal testing is only valid during the first trimester of pregnancy.
5. An advantage of DNA-based non-invasive prenatal testing is that the detection rates are the same for all forms of foetal chromosomal aneuploidies.
6. Discordant findings between the analysis of amniocytes and that of circulating cell-free foetal DNA could occur.
7. The foetal DNA fraction of a sample is an important parameter because inadequate foetal DNA in a sample could result in false-negative detection.
8. Confined placental mosaicism is a rare event and therefore needs not be considered in the interpretation of DNA-based non-invasive prenatal testing results.
9. Maternal karyotype abnormalities would not influence the DNA-based non-invasive prenatal test results.
10. Maternal occult malignancies could occasionally present as an incidental finding from non-invasive prenatal testing.

ANSWER SHEET FOR OCTOBER 2015

Please return the completed answer sheet to the Federation Secretariat on or before 31 October 2015 for documentation. 1 CME point will be awarded for answering the MCHK CME programme (for non-specialists) self-assessment questions.

Chapter 1
Non-invasive prenatal testing – a breakthrough in prenatal diagnosis

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Answers to September 2015 Issue

Breast Health

1. F **2.** F **3.** F **4.** T **5.** T **6.** F **7.** T **8.** F **9.** F **10.** T

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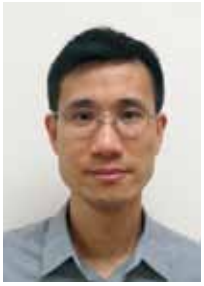
Chapter 2A
Debate: NIPT as primary screening for Down syndrome - FOR



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Introduction

Non-invasive prenatal testing (NIPT) can be used as a first-tier screening test or second-tier for cases screened positive using conventional screening methods. Currently, the major professional guidelines[1-6] all endorse NIPT as a secondary test for Down screening. The majority of them endorse it for primary screening for selected women at increased risk of having a Down foetus, such as maternal age 35 years or above, history or family history of having any Down baby, and being carrier of at-risk chromosomal aberrations. No professional body endorses it as a universal first-tier test yet. The International Society of Prenatal Diagnosis (ISPD) did recognise that some of its members were advocating NIPT as a universal first-tier test and women may choose to personally finance the test of their choice. In fact, to keep up with the rapid development in the subject, frequent revisions of these guidelines are anticipated. ISPD revised their 2011 position statement in 2013, within just 2 years of its release. Likewise, the Israel Society of Medical Genetics (ISMG), when drafting their committee opinion in late 2013, already planned for a revision within 1 year. The rapid development in NIPT ensures that by the time a guideline is released, it is already about time for a major revision. There are three main concerns using NIPT as a first-tier test universally: (1) test performance in a low- or mixed-risk obstetric population; (2) potential loss of other benefits offered by the current Down screening programme; and (3) the relatively high cost of NIPT.

Test performance in general obstetric population

NIPT has excellent performance in a routine obstetric population. Since the first study in low-risk women in 2012, today there are at least 12 large studies involving more than 1000 women each on the performance of NIPT for Down screening for low-risk pregnant women[7-18]. The total number of women studied amounts to 64,000. They all showed that the no call rate is extremely low (1.2-4.8% on 1st sample and 0.0-1.9% after redraw). The detection rate is >99.9%, comparable to that in the high-risk group. The false positive rate was ≤0.3%, comparable to that in the high-risk group, and was much lower than that of current screening strategies (false positive rate 4%). The positive predictive value is 46-91%, again many folds higher than that of current methods in use (positive predictive value 4.2%). In fact, the American College of Medical Genetics (ACMG) remains silent on the subject of defining candidates for NIPT, leaving open the possibility of using it with low risk women[19]. The organisation's rationale is that pregnant women should have access to the best screening test for common aneuploidies.

"Loss of other benefits of current Down screening programme"

The current Down screening programme sometimes detects chromosomal abnormalities unrelated to the initial indication. Some are worried that these lesions may be missed by targeted NIPT. In fact, these conditions do not fulfil criteria for screening. Many of them are randomly distributed and are not more common with positive Down screening results. They are picked up simply due to a higher false positive rate of the current Down screening programme and therefore more invasive diagnostic procedures are performed. It is the downside, and not an additional benefit, of current Down screening methods.

In fact, atypical autosomal aneuploidies are rare after 12 weeks because they are lethal beyond the first trimester. Why bother then? The phenotypes of sex chromosome abnormalities and other autosomal aberrations are variable, usually mild. Findings of unclear significance sometimes arise secondary to false positive Down screening results. These conditions cause complex counselling issues, especially in the absence of ultrasound abnormalities. They unnecessarily overload the highly-sought genetic counselling service. There is a significant ethical issue as well. Adequate pretest counselling is impossible given the multitude of possibilities associated with a false positive Down screening result. It poses potential psychological harm to the woman due to unpreparedness, anxiety and shock. Knowing more is not necessarily a blessing. To avoid this pitfall, the UK National Screening Committee has wisely recommended QF-PCR for confirmation of positive Down screening results[20].

Coupled with maternal characteristics, blood pressure and uterine artery Doppler, the current Down screening programme has the potential to predict development of pre-eclampsia and small babies. However, the need for multiple markers means individual markers are not good enough. If the current programme is replaced by NIPT, only the biochemical markers are lost. This is no big deal as biochemistry is not a good marker anyway.

Therefore, nothing is missed by switching to targeted NIPT. In fact, it helps to alleviate the problems caused

by the much higher false positive rate of current screening methods. Mind you that NIPT is not a replacement for quality prenatal ultrasound. Hand in hand, the two are complementary.

Cost

Cost-effectiveness analyses should be interpreted with extreme caution. When NIPT is used as a second tier test, the risk cutoffs to define high-risk group eligible for NIPT differ widely in different studies. The eligibility for NIPT as a secondary test is subject to manipulation. This has raised significant ethical concerns. Cost-effectiveness analyses are hypothetical, subject to extensive mathematical modelling and uncertainties. The analyses are flawed by the variable assumptions made and incomplete inclusion of all the costs. Discrepancy in the assumptions made and in cost inclusion has led to conflicting results in two recent Australian cost-effectiveness analyses of NIPT[21]. Assessment of psychological and non-monetary costs and benefits is challenging and many a time omitted.

Today, there are seven studies[21-27] attempting to address the cost-effectiveness issue of using NIPT for universal screening. The unit cost of NIPT quoted varied considerably. NIPT for universal screening was compared to its use for secondary screening, to its use in a hybrid approach, and to current screening strategies. NIPT for universal screening has consistently the best performance, but is the most costly, making it the least cost-effective in most studies. However, only two of these studies took into consideration the lifetime cost of missing a case of Down syndrome. The study by Evans et al[24] did not consider the non-medical cost of Down syndrome, nor pregnancy termination and miscarriage related costs. The study by Walker et al[22] attempted to include all costs to all parties in what they called a societal perspective. They came to the conclusion that NIPT is more effective and less costly than the Integrated Test currently in use, as long as the unit cost of NIPT is below USD549. Currently, two companies are offering NIPT at as low as USD500. Therefore, replacing the Integrated Test with NIPT is potentially cost-effective. The Belgian analysis[26] estimated that when the unit cost of NIPT was USD 190, it was cost comparable to replace the current Down screening programme (mainly first trimester combined screening based) by NIPT. Sequenom (SanDiego, CA) has announced the introduction of a low cost NIPT at USD 250-300 by the end of 2014. In fact, one major NIPT provider in China conceded that profit was made offering NIPT at around USD160 (personal communication). Therefore, replacing the current first trimester combined screening with NIPT at no additional costs is economically feasible.

Further falls in NIPT cost is expected for good reasons. Number one is advances in technology. Chromosome-selective sequencing, semiconductor sequencing and microarray-based analysis all have good potential to reduce costs compared to massively parallel sequencing. Upcoming is the revolutionary third generation sequencing, or nano-sequencing. Number two is the economics of scale attributed to increasing uptake of NIPT. Number three is price negotiation with government participation, through incentive structure, regulations and reimbursement policies. Number four is competition. Today, there are at least 13 NIPT providers worldwide. Three more are forthcoming in the USA. The competition is keen. Almost all NIPT providers in the US are embattled in lawsuits over enforcement and infringement of patents. In a recent case, the court invalidated the "540 patent" and denied Sequenom's request for injunction against Ariosa Diagnostics (San Jose, CA). Anyway, even if not invalidated, the "540 patent" will expire by 2017, paving the way for further reductions in NIPT cost. Therefore, cost is not an issue any more for universal Down screening using NIPT.

Equity of Access

From an ethical point of view, there is also strong ground for NIPT for all and not just for the selected few. If NIPT is an important and beneficial technology, it should be available to all patients[28].

We are not alone in the pursuit for NIPT for all. In a recent survey of members of the Society of Maternal Fetal Medicine (SMFM) in the USA, over half believed NIPT will be used instead of conventional screening procedures in all pateints[29]. In another survey of members of the American College of Obstetricians and Gynecologists (ACOG), the majority (79.1%) were of the view that NIPT should be offered to all patients, similar to current Down syndrome serum and ultrasound screening[30]. A recent survey in the UK confirmed that women share the same view[31].

Conclusion

The Belgian Health Care Knowledge Center (KCE) is a semi-governmental institution consisting of experts coming from different disciplines, including medicine, economics, statistics, sociology, psychology and law. A conclusion drawn by KCE[26] in May 2014 after reviewing the topic stated that NIPT for primary screening would be a most logical approach. For its high cost, it had been first positioned as a triage test (contingent or second line). A transition from triage to primary screening NIPT is to be planned when NIPT price allows this. Now, it does. Why wait then?

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Fragile X Syndrome

What is Fragile X syndrome?

Fragile X syndrome (FXS) is the most common known cause of intellectual disability (formerly referred to as mental retardation) that can be inherited, that is passed from parent to child. It is estimated that FXS affects about 1 in 4,000 boys and 1 in 6,000 to 8,000 girls. Both boys and girls can have FXS, but girls usually are more mildly affected.

What causes FXS?

The cause of FXS is genetic. FXS occurs when there is a change in a gene on the X chromosome called FMR1. The FMR1 gene makes a protein needed for normal brain development. In FXS, the FMR1 gene does not work properly. The protein is not made, and the brain does not develop as it should. The lack of this protein causes FXS. Other Fragile X-associated Disorders (FXDs) can be present in the extended family, even if not currently evident. Talk with a genetic counselor for more information.

What are some signs of FXS?

Children with FXS might:

- Sit up, crawl, or walk later than other children
- Have trouble with learning and solving problems
- Learn to talk later, or have trouble speaking
- Become very anxious in crowds and new situations
- Be sensitive about someone touching them
- Bite or flap their hands
- Have trouble making eye contact
- Have a short attention span
- Be in constant motion and unable to sit still
- Have seizures

Some children with FXS have certain physical features such as:

- A large head
- A long face
- Prominent ears, chin, and forehead
- Flexible joints
- Flat feet
- Macroorchidism (enlarged testicles in males; more obvious after puberty)

These physical features tend to become more noticeable as the child gets older.

What conditions are common among children with FXS?

Children with FXS might have learning disabilities, speech and language delays, and behavioral problems such as attention-deficit/hyperactivity disorder (ADHD) and anxiety. Some boys can develop aggressive behavior. Depression can also occur. Boys with FXS usually have a mild to severe intellectual disability. Many girls with FXS have normal intelligence. Others have some degree of intellectual disability, with or without learning disabilities. Autism spectrum disorder (ASD) occurs more often among children with FXS.

A New PCR-based Assay For Fragile X Testing

Workflow

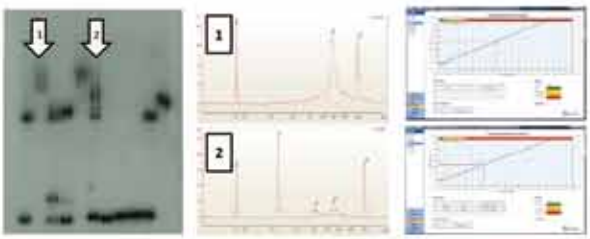

Figure 1. Two example cases representing representing full mutations samples are shown for a typical Fragile X Southern Blot together with the Bioanalyzer electropherogram and FraXsoft report after DNA amplification with FragilEase™.

By use of proprietary FragilEase that allows an accurate amplification of the trinucleotide repeats, combined with Caliper LabChip MultiDx instrument and FraXsoft ™, FMR I allelles can be reliably detected.

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CDC

Chapter 2B
Debate: NIPT as primary screening for Down syndrome - AGAINST

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Introduction

In Hong Kong, the universal first trimester combined screening (FTS) using foetal ultrasonographic measurements of nuchal translucency (NT) and serum biochemical markers to detect common aneuploidies has been implemented since 2010. However, since 2011 when non-invasive prenatal testing (NIPT) for aneuploidy using cell-free DNA (cfDNA) in maternal plasma came into clinical use, this has resulted in tremendous changes in our prenatal counselling and testing. Prospective and retrospective studies have shown high detection rates (DR) and low false positive rates (FPR) in the detection of common autosomal aneuploidy, mainly trisomy 21 (T21 Down syndrome) and trisomy 18 (T18), not just in the high risk group[1,2] but also in the low risk general population[3,4]. Thus, there are extensive discussions on how to implement this test into our clinical practice and whether NIPT should replace current FTS as primary screening.

Before any recommendation on the optimal implementation of NIPT could be made, the potential benefits and trade-offs of NIPT as primary screening compared with conventional FTS, and the cost-effectiveness analysis in cases of NIPT as primary screening have to be reviewed. Moreover, comparison of different screening models like contingent or sequential screenings against primary screening should also be considered.

1. The potential losses and benefits from NIPT compared to current universal FTS

Detection Rate

The conventional FTS can detect about 90% of cases with trisomy 21 (T21) and 95% of trisomies 18 (T18) and 13 (T13), at a FPR of about 5%[5,6]. Whereas in the latest meta-analysis of NIPT which identified 37 studies that included studies using cfDNA testing on both high risk and low risk populations using different approaches (massive parallel sequencing, targeted sequencing or single-nucleotide polymorphisms), suggested that the performance of screening for trisomy 21(Down syndrome) is superior, with higher DR of 99.2% and lower FPR of 0.09%[7]. Compared with the data published in a large local prospective audit, although our performance of FTS was proven to be as promising as the data published worldwide with a DR of 91.2% for trisomy 21, at a FPR of 5.1%[8], yet NIPT could potentially detect more trisomy 21.

However, the fact that the current combined screening is not just able to detect trisomy 21 but all three common trisomies and some other "atypical" chromosomal abnormalities, the limitation of current NIPT is that these "atypical" but potentially serious abnormalities could be missed if it is offered as primary screening.

The performance of NIPT in screening for T13 and T18 are worse than that of T21, with the DR of 96.3% for T18 which is just comparable to conventional FTS, and 91% for T13 which is lower than FTS at the FPR of 0.13% for both[7]. Cases with T13 are more likely to be missed if NIPT is offered as the primary screening. Although T13 and T18 are frequently associated with structural abnormalities which could be potentially "re-identified" by foetal structural scans, the truth is foetal structural scans are not routinely offered to our pregnant women in Hong Kong and this safety net does not exist.

Moreover, "atypical" chromosomal abnormalities are not uncommon in those screened high risk cases from FTS. Alamillo et al[9] reported that 30% of those screened as high risk have chromosomal abnormalities other than trisomies 13, 18 and 21 which included not just sex chromosomal anomalies but also chromosomal rearrangements, deletions and mosaicisms. The Danish group reported a similar figure of 23.4%[10]. These chromosomal abnormalities will be missed if NIPT is offered as the primary screening instead of the conventional one.

Although cfDNA analysis of maternal blood has now been extended for screening of sex chromosome abnormalities, its DR and FPR are much worse than that for trisomy 21 (DR 90-93%, FPR 0.37%)[7]. This could be multifactorial with related to the maternal and foetal sex chromosome mosaicism, the small informative part of the Y-Chromosome, the maternal age-related X-chromosome loss and the inherent sequencing bias associated with genomic guanine cytosine composition of the X-chromosome. Whether to screen for sex chromosomal anomalies could be debatable, but certainly these abnormalities still carry health and reproductive implications and it would be an individual judgement on its importance.

Moreover, even excluding those cases with sex chromosome abnormalities, 15-25% of other chromosomal abnormalities will still be missed by NIPT[9-10]. The prevalence of these "atypical" chromosomal anomalies was found to be increased in women above 45 years old, foetal NT thickness ≥3.5mm, abnormal levels of free β-human chorionic gonadotropin (<0.2 or ≥ 5.0 multiples

of the median (MoM)) or pregnancy-associated plasma protein-A<0.2 MoM[10]. Local data from the Prenatal Diagnosis Laboratory of The Chinese University of Hong Kong revealed that 25% of screened high risk cases from FTS which confirmed to have chromosomal abnormalities have other "atypical" abnormalities instead of common trisomies, and 55% of them were associated with high NT (personal communication). This confirmed the importance of the current established combined FTS with foetal ultrasound and maternal biochemistry, in selecting pregnant women who will benefit most from direct invasive procedures even in the era of NIPT.

Furthermore, with the advance in technology, nowadays invasive procedures with the option of microarray testing allow the detection of a broad range of additional abnormalities not yet detectable by conventional karyotype or NIPT[11,12]. A prospective study conducted by the National Institute of Health (NICDH) revealed that in cases with ultrasound abnormality and normal karyotype, 6% were found to have clinically relevant copy number variations (CNV) using microarray[13].

Although historically the screening programme was targeted on Down syndrome, nowadays the conventional FTS followed by invasive tests in high risk cases has improved the screening performance in providing a more comprehensive foetal assessment. Together with the array technology, much more valuable information concerning foetal well-being can be ensured compared with using NIPT as primary test. The improved DR of Down syndrome from 91 to 99% is achieved at the expense of missing other "non-T21" abnormalities when NIPT is offered as primary screening.

The issue of NIPT in multiple pregnancies is even more complicated because of the possible genetic discordant and the different contributions of foetal fractions in the maternal plasma. In general, the performance of NIPT in multiple pregnancies is less well established than a singleton pregnancy.

False positive and no-result rate

The other proposed benefit from the NIPT is the potential reduction of invasive procedures and the procedure related pregnancy loss with respect to its low FPR. Despite the very low FPR from screening of T21, the cumulative FPR from the NIPT has to include those from screening of T13 and T18 which will be 0.35%. If screening for sex chromosome abnormalities is included, the FPR will further increase to 0.72%[7].

Moreover, another drawback of adopting NIPT as primary screening is its failure to provide a result. The reported "no-result" rates from the latest meta-analysis ranged from 0.0% to 12.2%[7]. There were multiple possible reasons with regard to the failure including problems in sample collection and transportation, laboratory assay failure, but more importantly was the low foetal fraction in the maternal plasma which could be affected by the gestation and maternal weight[14]. The low foetal fraction was reported as the reason of failure in 0.5-6.1%[7]. Despite redraws and retests, persistent failure existed and it has been reported as high as half of the cases in the redrawn group in one study[15].

In view of the very wide range of reported failure rate and the heterogeneity of those studies in the meta-analysis, the author commented that no conclusion can be made to pinpoint the reason or particular methodology is the culprit for failure. However, it certainly reflected the importance of quality control which could significantly affect the failure rates and performance. The higher laboratory failure rate from NIPT testing comes from those including sex chromosome. But even if that has been excluded and inadequate samples were omitted, the laboratory failure rate still ranged from 0-6.3%. Nonetheless, the repeated testing and failure will cause delays in management and the pregnant women will lose their opportunity to go back for the conventional FTS and ended up with either no risk assessment, or having to undergo the less sensitive second trimester biochemical screening (DR 70%), or direct invasive diagnostic tests. A recent large prospective, multicentre, blinded study conducted at 35 international centres has revealed a 3% "no-result" rate and the prevalence of aneuploidy in this group (2.7%) is higher than the prevalence (0.4%) in the overall cohort[16]. There is no reliable way to predict how pregnant women with failed NIPT will react and choose. But if they opt for an accurate test as comparable to NIPT, direct invasive tests will be necessary. By adding up the FPR and no-result rate, the potential cases require invasive tests after NIPT can be as high as 4% which is not much different from the FPR from conventional FTS.

2. Cost

The other major limitation of NIPT as primary screening is the high cost of the test. There were multiple cost-effectiveness analyses published which showed that the marginal cost of NIPT as primary screening is exceeding the lifetime cost of a Down syndrome birth. It is understandable that the different analysis models based on different local circumstances, assumptions, local costs of tests and different inclusions in the analysis, would lead to different results and these models may not apply to our locality. However, it is important to know nearly all of these analyses consistently commented that implementing NIPT as primary screening is unlikely cost-effective or only be feasible if the costs of NIPT decreases dramatically[17-20]. The conclusion was made even when the costs from missing those cases with other chromosomal abnormalities were not included. The study published by Song et al. concluded that NIPT could be cost-effective as primary screening, however in their analysis the detection rate of Down syndrome by FTS was only 85% which is much lower than our performance and the majority of the costs in their analysis was actually coming from those not received any screening but not missing from the FTS[21]. Their analysis obviously could not apply to ours. The potential cost of each additional case of Down syndrome being detected was estimated to be HK$18M[22]. It is also important to remember that termination of pregnancy for those confirmed with Down syndrome is not the only option and a proportion of women will continue the pregnancy. The parent's choice of continuing pregnancy has not been counted in which the cost of preventing one Down syndrome birth will be even higher.

More importantly, we have to compare the different potential screening models and select the one that can provide a good and comprehensive screening

performance, and being more cost-effective. Continuation of the conventional FTS as primary screening followed by invasive procedures for those high risk cases could provide more comprehensive foetal assessment without missing those "atypical" chromosomal abnormalities. Adding NIPT as contingent screening for those with intermediate risk can improve the detection rate for T21 with much lower cost. The majority of the studies mentioned above favoured the implementation of NIPT as a contingent screening from the cost-effectiveness point of view as well. In cases of failed NIPT, pregnant women can also rely on the results of the FTS to decide for invasive tests or not. This may be a more suitable model based on the current cost and performance, and if resources are allocated to improve the detection of Down syndrome.

Conclusion

In conclusion, although NIPT as primary screening can improve the detection rate of Down syndrome, it will miss out more other foetal chromosomal abnormalities compared to current screening and it is not cost-effective. The apparent benefit of reduction of miscarriages may have been overestimated. Therefore, NIPT should not be implemented as primary screening to replace the current strategy. This conclusion is supported by the recent Committee Opinion written by the American College of Obstetricians and Gynecologists and the Society of Maternal-Fetal Medicine[23].

To screen for Down syndrome is only part of our antenatal care. Our ultimate goal should aim to provide a more comprehensive foetal and maternal assessment. Nowadays the first trimester combined screening has moved forward for early foetal structural assessment and even antenatal complications screening like pre-eclampsia and preterm labour. Incorporating NIPT into the current universal FTS as contingent screening, on one hand, will gain the benefit of improving the detection rate for T21, and on the other hand, it allows the obstetricians to triage and counsel those women who will benefit from invasive tests with the option of microarray. This model is likely to be a more cost-effective way to improve our services.

The technology in NIPT is rapidly expanding and evolving. There are reported uses of NIPT in detecting microdeletion or microduplication syndromes. It is anticipated that NIPT will eventually not just be used for testing for foetal common trisomies, but also for a broader range of other genetic disorders. The cost of the test will likely drop with time as well. Therefore, re-evaluation of the optimal implementation such as used as combined testing will be required when these can be achieved.

Last but not least, it is important to understand how easily the test quality can compromise the performance. There were reported flaw results of normal NIPT with foetal fraction detected even in non-pregnant women[24]. Irrespective to conventional FTS or NIPT, contingent or primary screening, quality control is the crucial factor to ensure its optimal performance. NIPT should not be used as a "simple" blood test like part of the routine antenatal blood. Adequate pretest counselling and appropriate foetal assessment by trained personnel/obstetricians and foetal medicine specialists are essential to guide the optimal screening and actions for the individuals. Otherwise, instead of improvement, there would be deterioration in the care delivered to our pregnant women despite the breakthrough in technology.

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Chapter 3
Role of chromosomal microarray in prenatal diagnosis

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Introduction

Chromosome analysis has been the gold standard for detecting chromosomal abnormalities in prenatal diagnosis. It enables genome-wide detection of numerical and structural abnormalities at a resolution of 5–10 Mb[1]. The method is labour intensive, with a turn-around time of about two weeks, requiring cell culture, metaphase preparation, and karyotyping by trained cytogeneticists. Other molecular techniques developed for rapid aneuploidy and micro-deletion/duplication detection, such as quantitative fluorescent PCR (QF-PCR), multiplex ligation-dependent probe amplification (MLPA), and BACs-on-Beads (BoBs), offer shorter turn-around time, but target only specific and limited number of genomic loci. In contrast, molecular karyotyping using chromosomal microarray (CMA) provides both a rapid and high resolution genome-wide screening for genomic imbalances or copy number variants (CNV).

What are chromosomal microarrays?

Chromosomal microarrays detect gain and loss of genomic regions by hybridisation of fluorescently labelled test DNA from a patient (foetal sample) onto targets with known genomic coordinates, which are usually fixed on a glass slide. By comparing the signal intensity of the patient and control DNA, chromosome gain or loss can be identified by computerised systems.

Chromosomal microarray can be performed by different platforms. It can be a BAC (bacterial artificial chromosomes) array, which uses probes of DNA constructs of size 150-750 bp. More commonly over the years, CMA, namely oligonucleotide array comparative genomic hybridisation (oligo aCGH) with size of oligonucleotide probes ranging 25-75 bp, is preferred. This allows detection of chromosome gain and loss at a higher resolution than BAC array. Single-nucleotide-polymorphisms (SNP) arrays, in addition to detection of chromosome gain and loss, can give information on loss of heterozygosity, with detection of uniparental disomy[2].

The design of the CMA can be genome wide, with probe coverage of the whole genome, or custom-designed, as targeted CMA, with probe coverage only on clinically significant genomic regions. Whole genome CMA, with higher coverage across the genome, can help to delineate breakpoints and characterise marker chromosomes and de novo or cryptic imbalances on top of targeted regions with clinically relevant imbalances. While the use of targeted CMA may reduce detection of variants of unknown significance (VOUS), with rapid increase in knowledge on CNV and new microdeletion/duplication syndromes, targeted CMA may need frequent updates.

The advantages of CMA compared to conventional cytogenetics include increase in resolution of chromosome analysis in detection of submicroscopic gain or loss, a shorter turn-around time because cell culture is not required, and capability of high throughput processing as it is less labour intensive [Table 1]. Systematic reviews had shown an increased diagnostic yield of CMA of 6-10% for foetuses with ultrasound abnormality and normal karyotype, while the detection of VOUS remains low at around 1-2%[3-5]. Limitations of CMA include inability to detect structural rearrangement of chromosomes like balanced chromosomal rearrangements. Copy number variations not represented on the array design will be undetected. Low level mosaicism or maternal contamination may also be difficult to detect, depending on the CMA platform used. Triploidy and uniparental disomy cannot be detected, except using SNP arrays; however, these two kinds of chromosomal abnormalities can be detected by other rapid molecular detection methods, such as QF-PCR.

Table 1: Comparison of conventional cytogenetics and CMA

	Conventional cytogenetics	CMA
Sample requirement	Cultured cells	DNA
Resolution	5-10 Mb	200 kb or less
Turnaround time	2-3 weeks	1 week
Laboratory requirement	Labour intensive	High throughput
Detect balanced rearrangement	Yes	No
Detect triploidies	Yes	No (for oligo aCGH)

How are prenatal CMA findings or CNVs interpreted?

Copy number variants interpretation and reporting in the postnatal setting are well defined[6]. However, the interpretation of CNV in the prenatal setting can be more challenging because of the limitation of phenotype information from ultrasound examination. In general, the clinical significance of CNV depends on its size, its gene content, evidence on haploinsufficiency/triplosensitivity, inheritance of the CNV, any previous reports, and relevance between the disrupted gene and phenotype. In general, whole genome CMA enables detection of copy number variants at size in the region of <200 kb at the backbone and at smaller sizes at disease-focused regions.

CNVs detected are systemically evaluated for their clinical significance. They are compared against the publicly available databases that collect data from healthy individuals and from individuals with multiple congenital anomalies and developmental disabilities. The following are the well-known international databases: the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation), Copy number variation project database at the Children's Hospital of Philadelphia (CHOP, http://cnv.chop.edu), Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER, http://decipher.sanger.ac.uk), the International Standards for Cytogenomic Arrays (ISCA) Consortium database (ISCA, http://dbsearch.clinicalgenome.org), and Singapore Database for Copy Number Variants (http://www.statgen.nus.edu.sg). Published in-house datasets[7,8] can be used to compare array results and documented phenotypes. Communication with well-established genetics laboratory partners providing aCGH testing service, referring clinicians and clinical geneticists is important to render comprehensive analysis for interpretation and reporting.

Generally, CNV are categorised into three types in the prenatal setting: clinically significant, unclear clinical significance, or benign with the following results:

(1) Normal molecular karyotype: no known syndrome-associated imbalance or CNV of unknown significance is detected.

(2) Clinically significant CNV: a chromosome imbalance harbouring genes and/or overlapping with a known syndrome which is clinically well defined or reported in the literature, for example, described in Online Mendelian Inheritance in Man (OMIM) database , or with an entry in public databases, such as DECIPHER, ISCA, and ECARUCA. The region is not noted as a variant found in healthy individual(s) in the public database. To investigate whether the clinically significant imbalance is familial or de novo, parental study is advised.

(3) CNV of unclear clinical significance: a chromosome imbalance, which has not been reported in literature or noted in the public/in-house databases, is detected. It is not possible to predict the phenotypic effect prenatally. Inheritance of this CNV from apparently unaffected parents may not preclude pathogenic effects because of possibility of incomplete penetrance and variable expressivity. Counselling by a clinical geneticist is advised.

A prenatal case to share

The following example illustrates the benefit of oligo aCGH as well as the complimentary use of conventional and molecular cytogenetic techniques in prenatal diagnosis, allowing rapid detection of a chromosomal syndrome carrying a grave prognosis.

A 29 year-old woman of good past health had her first pregnancy. There was no family history of congenital abnormality. She had first trimester combined Down syndrome screening which showed a low risk result. Morphology scan at 21 weeks showed bilateral cleft lip and cleft palate. There were no other structural abnormalities and the foetal parameters were 1 week smaller. The couple were counselled on the prognosis of isolated versus syndromic cleft lip and palate. Amniocentesis was performed for oligo aCGH and karyotyping. Oligo aCGH on uncultured amniocytes showed a 8.7 Mb terminal deletion in the short arm of chromosome 4 (4p) and a 6.75 Mb terminal duplication in the short arm of chromosome 8 (8p) (Figure 1). The deleted 4p region contained 111 genes including the critical region for Wolf-Hirschhorn syndrome (OMIM 194190). This is a congenital malformation syndrome characterised by pre- and post-natal growth deficiency, developmental disability of variable degree, characteristic craniofacial features, and seizures. The oligo aCGH result was available to the couple in 5 days for them to make a decision, after counselling, on termination of pregnancy. The abortus had bilateral cleft lip and palate. Conventional cytogenetic study on cultured amniocytes showed 46,XY,der(4)t(4;8)(p16;p23) by day 15. The abnormal chromosome material on the terminal short arm of chromosome 4 was confirmed to be material translocated from chromosome 8 by multicolor-FISH (mFISH) study (Figure 2). Further testing on the couple's blood showed normal karyotype. Hence, it is a de novo change in the foetus, with no increased recurrence risk for future pregnancy.

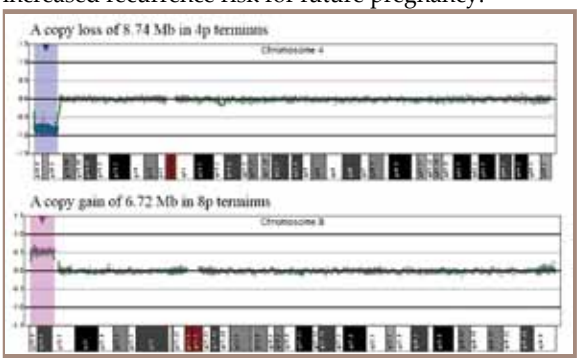

Figure 1. Oligo aCGH results of uncultured amniocytes with a 8.7 Mb terminal deletion (blue dots and light blue colour shaded region) in the short arm of chromosome 4 (4p) and a 6.75 Mb terminal duplication (red dots and pink colour shaded region) in the short arm of chromosome 8 (8p). Plot of $\log_2$ ratio of oligonucleotide probes fluorescent signal intensity on chromosomes 4 and 8.

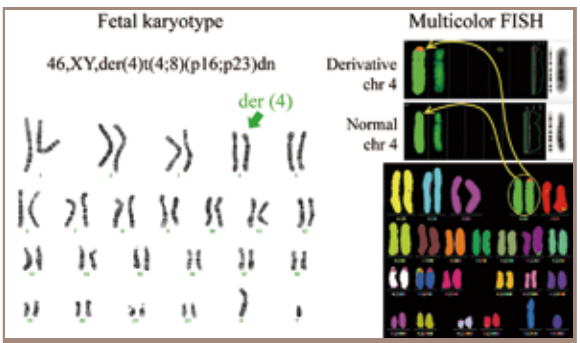

Figure 2. Conventional cytogenetic study and mFISH investigation on cultured amniocytes. Left panel: foetal karyotype of cultured amniocytes was 46,XY,der(4)t(4;8)(p16;p23)dn. The derivative chromosome 4, der (4), was confirmed to be de novo after parental karyotype investigation. Right panel: mFISH result showed that the abnormal chromosome material (orange colour) on terminal short arm of chromosome 4 was material translocated from chromosome 8. The orange colour segment corresponds to chromosomal material from chromosome 8. Two small insert images (shown on the top) show magnified normal and derivative chromosome 4.

Are there guidelines and recommendations on prenatal aCGH?

The most recent published recommendations from the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine in 2013 endorsed the use of CMA instead of karyotyping for foetuses with ultrasound abnormalities, as well as for low risk population regardless of age. Its use is also recommended in intrauterine foetal demise and stillbirth (Table 2) [9].

Table 2: Recommendations for the use of CMA analysis in prenatal diagnosis by the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine in 2013.[9]

- In patients with a foetus with one or more major structural abnormalities identified on ultrasonographic examination and who are undergoing invasive prenatal diagnosis, CMA analysis is recommended. This test replaces the need for foetal karyotype.
- In patients with a structurally normal foetus undergoing invasive prenatal diagnostic testing, either foetal karyotyping or a CMA analysis can be performed
- Most genetic mutations identified by CMA analysis are not associated with increasing maternal age; therefore, the use of this test for prenatal diagnosis should not be restricted to women aged 35 years and older.
- In cases of intrauterine foetal demise or stillbirth when further cytogenetic analysis is desired, CMA analysis on foetal tissue (i.e., amniotic fluid, placenta, or products of conception) is recommended because of its increased likelihood of obtaining results and improved detection of causative abnormalities
- Limited data are available on the clinical utility of CMA analysis to evaluate first-trimester and second-trimester pregnancy losses; therefore, this is not recommended at this time.
- Comprehensive patient pretest and posttest genetic counselling from qualified personnel such as a genetic counsellor or geneticist regarding the benefits, limitations, and results of CMA analysis is essential. Chromosomal microarray analysis should not be ordered without informed consent, which should be documented in the medical record and includes discussion of the potential to identify findings of uncertain significance, non-paternity, consanguinity, and adult-onset diseases.

The American College of Medical Genetics has also set up guidelines for application of CMA for prenatal diagnosis in the same year[10]. Recommendations for using CMA or aCGH for prenatal diagnosis were also made in other countries, such as Canada[11], Italy[12] and Belgium[13].

What are the prerequisites for obstetricians offering aCGH in prenatal diagnosis?

With the increase in utilisation of advanced technology like CMA in prenatal diagnosis, it is important for health care providers to be equipped with knowledge and skills in counselling and offering support and appropriate referral for these couples pre and post test. A previous survey conducted among local doctors and antenatal patients showed that in general aCGH is perceived as a better test than conventional cytogenetics and nearly 70% patients would choose aCGH as the diagnostic test of choice. However, there is a gap in genetic counselling training among obstetricians and nurses that is necessary to provide improved doctor-patient experience in Hong Kong[14].

On adequate pretest counselling, the use of information sheet and diagrams to explain test details, indications, sample requirement, possible test results and its return, potential need of parental testing to clarify inheritance of CNV, limitations of test and other possible implications to family is advised (accessible on http://www.obsgyn.hku.hk/prenatal_diagnosis). The approach of allowing couples to indicate what test results they would like to know in the informed consent form has been reported[15,16] although more recent studies indicated most women like to know as much information as possible from test results . Hence, the ethical discussion on whether to report all information to respect patient autonomy or to report selected information to respect their right to 'not to know' is still ongoing[17,18].

Obstetricians who read reports issued from clinical laboratories are the first professional to do the initial post-test counselling for the majority of women with normal prenatal aCGH results, and the minority with results which are clinically significant, or of uncertain/unclear clinical significance. Close communication among obstetricians, laboratory scientists and clinical geneticists are of vital importance to enable comprehensive prenatal counselling on CNV which are clinically significant, or of uncertain/unclear clinical significance. In Hong Kong, as there is still no proper profession or post of genetic counsellor, referral to clinical geneticists for post-test counselling and discussion on complex CMA results or findings or uncertain clinical significance is required[19]. These prenatal consultations are always available and are well supported by the Clinical Genetic Service of the Department of Health, or clinical geneticists of the two local universities.

Can CMA replace conventional cytogenetics for prenatal diagnosis?

Various groups have demonstrated the clinical utilities[20,21] and approved the offering of CMA as an adjunct diagnostic tool in prenatal cases with foetal ultrasound abnormalities[9,11,12]. It has been demonstrated that combining rapid aneuploidy detection test, such as QF-PCR, CMA would be an effective first-tier prenatal testing regime that does not require conventional karyotyping[7,22].

In Hong Kong, CMA can be integrated into the existing Universal Down Syndrome screening programme, with foetal ultrasonography for prenatal diagnosis[7]. Anticipating the implementation of the highly sensitivity and accurate non-invasive prenatal screening (NIPS) on circulating cell free foetal DNA in maternal plasma, NIPS can also be incorporated into the model. As shown in Figure 3, women screened positive for foetal Down syndrome can be offered an option of having NIPS for foetal trisomy assessment if there is no ultrasound abnormality. In cases where ultrasound examination shows foetal abnormalities, an invasive diagnostic test could be performed for CMA.

Rapid aneuploidy detection (e.g. by QF-PCR) of the chorionic villus or amniotic fluid sample would exclude common aneuploidies, polyploidies and maternal cell contamination before CMA. Back up cultures should be set up for cytogenetic investigations, for cases with abnormal CMA results. Abnormal CMA results can be confirmed by karyotyping if the CNVs are large (>10 Mb). For small CNV, metaphase and interphase FISH (for CNV size 1-10Mb) or digital PCR for copy number quantitation (for CNV size <1Mb) can be performed for verification. Abnormal findings in rapid aneuploidy detection test need to be followed by conventional cytogenetic study to assess whether there is any parental balanced translocation to determine recurrence risk. This approach would reduce conventional cytogenetic testing by around 80%, as estimated by a local study[7].

and small heterochromatic marker chromosomes with non-gene coding material.

Conclusion

Chromosomal microarray has been widely evaluated in prenatal setting and adopted as a clinical diagnostic tool, as it provides increased diagnostic yield with shortened turn-around-time. With accumulated knowledge and experience, analysis and interpretation of data generated from CMA become less difficult. Obstetricians need to be aware of the importance and impact of pre and post-test counselling on couples. Until the development of more comprehensive genomic testing, obstetricians shall prepare to anticipate the replacement of conventional cytogenetics by CMA in prenatal diagnosis.

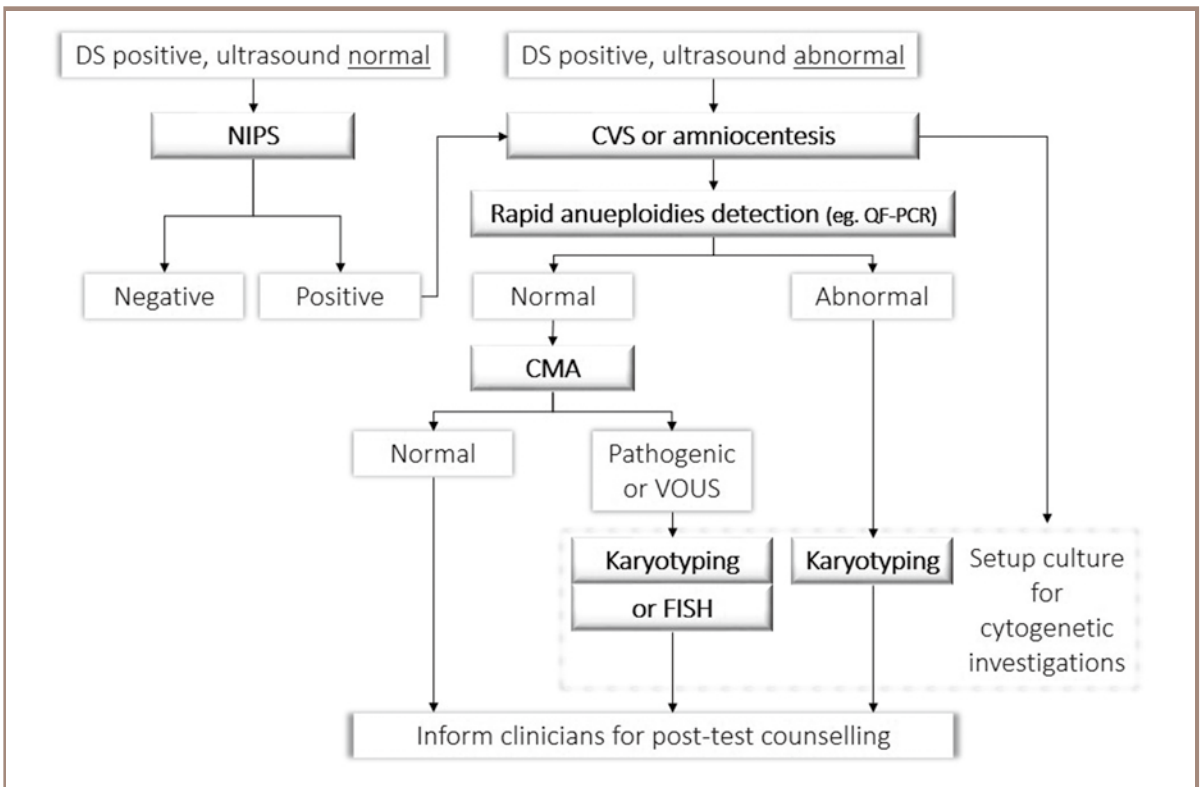

Figure 3. Proposed workflow for incorporating chromosomal microarray (CMA) in prenatal diagnosis in Hong Kong. Pregnancies with Down syndrome screening (DS) positive without ultrasound abnormalities can be subjected to non-invasive prenatal screening (NIPS) on circulating cell free foetal DNA in maternal plasma; while pregnancies with DS positive in the presence of ultrasound abnormalities can be subjected to chorionic villi sampling (CVS) or amniocentesis followed by rapid aneuploidy detection (e.g. QF-PCR) to exclude common aneuploidies and maternal cell contamination and then aCGH analysis. Cell culture will be setup for cytogenetic investigations, such as karyotyping and FISH.

Chromosomal microarray can be incorporated into the prenatal diagnostic service in Hong Kong, but would not entirely replace conventional cytogenetics, as cytogenetic expertise is needed for producing good chromosomal metaphases for validation of variants identified by CMA, and further investigation of CMA findings showing aneuploidy to see if the chromosomal imbalance has arisen from translocation, to assist counselling on recurrence. Laboratories planning to adopt the proposed strategy need to inform obstetricians that the strategy will not detect prenatally insignificant findings, including balanced rearrangements, low level mosaicism (sensitivity level would be platform specific)

Glossary

Bacterial artificial chromosomes (BACs): cloning vectors which allow insertion of pieces of fragmented human genome, approximately 150 kb in length, for amplification by bacteria.
BACs array: an array spotted with a selection of BAC clones which assemble the entire human chromosome complement.
Copy number variants (CNV): stretches of DNA larger than 1 kb that display copy number differences[23].
Multicolor FISH (mFISH): an ordinary fluorescent

in-situ hybridisation (FISH) analysis is usually locus specific, investigating cytogenetic origins of chromosomal aberrations using fluorescently labelled probes. Based on similar principle as in ordinary FISH, mFISH uses fluorescent labelled probe cocktails, which hybridise (or "paint") the whole set of chromosomes in metaphase. A specific fluorescent microscope equipped with multi-fluorescence excitation filters is utilised for image acquisition from which each chromosome will be distinguished by its unique combination of emitted fluorescence.

Oligonucleotide array: An array spotted with short synthesised DNA oligonucleotides as detection probes.

Single-nucleotide-polymorphisms (SNPs, pronounced "snips"): single nucleotide base pair changes, with one in every 300 nucleotide on average and roughly 10 million SNPs in the human genome.

SNP array: An array using immobilised allele-specific oligonucleotide (ASO) probes.

Variant of unknown significance (VOUS): rare or novel variant imbalance with undetermined pathogenicity. The effect of the imbalance to the referral indication is unclear. In some circumstances, there may be emerging evidence suggesting, but not yet fully confirming, the nature of the variant. In these cases, the variant may be classified as "likely benign" or "likely pathogenic". Parental CMA analysis may help classify a VOUS as "likely benign" or "likely pathogenic." A fetus inheriting a VOUS from a normal parent may reduce the chance that the variant is responsible for the referral indication and phenotype. The interpretation of a VOUS may change over time when more evidence emerges.

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H.K. Patent

U.S. Patent

China Patent

Source: 1. IMS 2010 Cord Blood Bank Market Research in Hong Kong (with Private O&G physicians) 2. ipsos Healthcare 2009-2015 Cord Blood Bank Survey
3. Introduction to the Cord Tissue Market and Cord Tissue-Derived Mesenchymal Stem Cells (CT-MSCs), BIOMEDPHARMIT, Jun 2015

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Chapter 4
Role of ultrasound in the new algorithm of prenatal diagnosis

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Introduction

Recently, molecular genetics hit the world of prenatal diagnosis with two amazing inventions – Non-invasive prenatal test (NIPT) by using maternal plasma cell-free DNA (cfDNA) and microarray-based comparative genomic hybridisation (aCGH). While the new technologies are taking up more pages and sessions in foetal medicine journals and conferences, one may query if ultrasound is losing its place in prenatal diagnosis.

USG in First trimester

Dating of pregnancy

Prenatal diagnosis starts with a viable pregnancy. Ultrasound has an un-replaced role in confirming the number, location, viability and gestation of a pregnancy. When a pregnant woman comes to the prenatal clinic, the first thing her obstetrician wants to know is the gestation. Unless the woman has regular 28-day menstrual cycles, ultrasound measurement of the foetal crown-rump length in the first trimester (between 8 and 13+6 weeks) is the most accurate method to establish or confirm gestation[1].

Why dating the pregnancy is important in prenatal diagnosis? The conventional first trimester Down syndrome screening consists of measuring the foetal nuchal translucency (NT) combined with maternal serum markers (beta-HCG and PAPP-A) to generate the risk ratio. The test is limited to the gestation 11-14 weeks when the foetal crown-rump length (CRL) lies between 45 to 84 mm. As NT increases with gestation, the Fetal Medicine Foundation suggests using the 95th percentile nuchal translucency value according to the CRL as the cut-off.

The recent development of NIPT measures cfDNA in the maternal serum for aneuploidy screening[2]. It extends the screening period outside the first trimester. However, the test should not be performed before 8 weeks gestation as it carries a higher risk of 'no result' because of the low foetal cell fraction in the maternal serum.

Down syndrome screening

For the conventional first trimester (11-14 weeks) screening test, the detection rate (DR) is about 90% for Down syndrome, 50% for trisomy 13 and 80% for trisomy 18; at a false positive rate (FPR) of 5%. Women who have 'positive' results would be offered invasive tests (i.e. choriovillous sampling or amniocentesis) for definitive diagnosis. In other words, 5% of the women would undergo unnecessary invasive tests that carry up to 1% of miscarriage risk.

Compared to the conventional tests, NIPT is undoubtedly more sensitive. The DR is over 99% for Down syndrome and trisomy 13, and around 90% for trisomy 18. The FPR is extremely low (<0.1%)[3]. Positive results need confirmation by invasive tests. Ideally, the number of 'unnecessary' invasive tests is markedly reduced. NIPT seems to be a 'near-perfect' screening test, however, this is not the truth. One important drawback of replacing conventional screening by NIPT is around 3% of patients would get 'no result' from the test because of the low foetal DNA fraction in the maternal serum. As the prevalence of aneuploidy in this group might be higher than the overall cohort (2.7% vs 0.4%)[3], whether invasive tests should be performed for the 'no result' group is still under discussion. Conventional screening might act as a backup for this group of patients.

Since neither test is ideal, the conventional screening test should still have a role in the new algorithm of prenatal diagnosis. We would like to suggest the following strategy:

If the woman presents at the first trimester, ultrasound examination would be performed for viability, number, dating of pregnancy and NT measurement. Information on the conventional screening and NIPT could be discussed. A cytogenetic test could be incorporated into the conventional screening by using NT as the triage:

1. The cut-off to 'high risk' in conventional screening is 1:250 (may have slight variations according to laboratories). However, most of the results are 'false positive'. If NT is normal and there is no abnormal ultrasound features, NIPT could be performed before considering invasive diagnostic tests. As NIPT has a high DR for Down syndrome, combination of two tests would markedly reduce the FPR. Fewer women would receive unnecessary invasive tests, which are associated with pain, infection and 1% risk of miscarriage.

2. As the DR of any conventional test is best at around 90%, one in 10 of the Down's pregnancy would be missed. Some institutions define the risk of 1:250 to 1:1200 as 'intermediate risk'. By principle, an invasive test is not justified. We suggest NIPT could be offered to this group. This might slightly increases the DR without increasing the number of unnecessary invasive tests.

Multiple pregnancies

In multiple pregnancies, early ultrasound to determine the number and chorioamnionicity is important. Monochorionic pregnancy carries higher risks and should be managed under different protocols. In contrast to singleton pregnancies, the conventional Down's screening for twin pregnancy is only based on the measurement of NT only, this lowers the detection rate to about 60%. In twin pregnancies screening by NIPT is feasible, but the failure rate is higher and the detection rate may be lower than singleton pregnancies. In the cumulative data from the literature in twin pregnancies, the DR for the trisomy 21, 13 and 18 were 95%, 100% and 86% respectively, at FPR of 0%[4]. There may be pitfalls in cases of a low foetal cell fraction from one of the foetuses.

Screening for congenital abnormality

NT measurement has another important role in triaging high risk pregnancies to more advanced tests in prenatal diagnosis. The association of thick NT with chromosome aneuploidies, structural abnormalities and genetic syndrome has been widely studied. The risk increased considerably when NT > 3.5mm. In a large study, the incidence of abnormalities was 46% in foetuses with NT > 6.5 mm[5]. In addition, the risk of major cardiac defects also increased progressively with thick NT. The risk was 3% for NT 3.5-4.4 mm, 7% for 4.5-5.4 mm, 20% for 5.5 -6.4 mm and 30% for NT >= 6.5 mm[4]. More than that, case reports and case control studies suggested that thick NT is associated with a large number of structural abnormalities and syndromes such as diaphragmatic hernia, omphalocoele, body-stalk anomaly, skeletal defects, congenital adrenal hyperplasia, foetal akinesia deformation sequence, Noonan syndrome, etc. Even if thick NT is an isolated finding, the risk of genetic disorders is also higher. Many of these are linked to submicroscopic chromosomal abnormalities that are typically missed by conventional karyotype.

Therefore, we suggest no matter whether NIPT is used as the primary or secondary screening test, NT should be measured if the gestation is appropriate. If NT is greater than 3.5mm, a direct invasive test for karyotype followed by a detailed structural scan at the second trimester is more appropriate than NIPT. If the karyotype is normal, aCGH should be the 'extended' test to be considered.

aCGH is a powerful tool in detecting imbalances in the clinically relevant genome. It could be a copy-number gain or copy-number loss – referred to as copy number variations (CNVs). This subtle change on the chromosome is too small to be detected by conventional karyotype, florescence in-situ hybridizsation (FISH) or polymerase chain reaction (PCR) technique. In a recent study, the risk of pathogenic CNVs was 5.3% in the group with thick NT (>3.5 mm) without other sonographic anomalies[6].

First trimester ultrasounds also provide important information on uterine anomalies, adnexal cysts, placental features and foetal anomalies. Early detection of major foetal anomalies is possible -- Some examples are acrania/anencephaly sequence, holoprocencephaly, cephalocoele, cystic hygroma, hypoplastic left heart syndrome, ectopia cordis, omphalocoele, gastroschisis, megacystis, abdominal cysts, major spine deformities, missing limb or limb reduction defects, etc. The detection rate varies with expertise. Earlier detection of major foetal anomalies enables earlier genetic diagnosis and easier termination of pregnancy if appropriate.

Screening for PET

An increased uterine artery Doppler pulsatility index (PI) > the 95th centile in the second trimester (16-24 weeks) is a good predictor of subsequent development of pre-eclampsia (PET) and foetal growth retardation (FGR) requiring delivery before 32 weeks (sensitivity 90.0% and 56.3% respectively). It has been used for a decade to identify the high-risk pregnancies for close monitoring. Recently, there are data on its application to 11-14 weeks gestation. The sensitivity is 60.0% for PET and 27.8% for FGR[7]. Despite the lower detection rate, the potential advantage of an earlier screening is to make prophylactic treatment by maternal use of low-dose aspirin before 16 weeks possible. More publications are awaited on the extent of benefits.

USG in second trimester

Down syndrome screening

At the second trimester (16-19 weeks), only the maternal serum biochemical markers (AFP, hCG, unconjugated E3, and sometimes inhibin A) are measured; the DR is about 65-80% for Down syndrome, at the FPR of 5%. Before the invention of first trimester Down's screening or NIPT, there have been various publications on using sonographic 'soft markers' to modify the risk, e.g., nasal bone hypoplasia, short long bones, choroid plexus cyst, echogenic foci in heart, echogenic bowel, mild pyelectasis, single umbilical artery, etc. Each of them carries different odds ratios. NIPT would help to relieve the 'iatrogenic anxiety' created from these 'soft markers'. Since conventional screening in the second trimester is less sensitive, direct NIPT for aneuploidy screening might be a reasonable option for pregnancy presented late at the second trimester.

Structural abnormality

Foetal anomalies occur in about 3% of pregnancies. They are diagnosed by ultrasound prenatally with increasing sensitivity and specificity. The second trimester '18-22 week' scan remains the standard for evaluation of foetal anatomy in both low-risk and high-risk pregnancies. There are recommendations on the requirement of basic routine scans and the extended views, which can be obtained if feasible, to improve the detection of foetal abnormalities[8,9]. Some examples of extended views are left and right outflow tract of the heart, three-vessel tracheal view of heart, sagittal facial profile, nose and ears, counting fingers and toes, etc. The prognosis of foetal anomalies is variable - it depends on the type of anomaly, whether it is isolated or being part of the malformation syndrome, and the underlying genetic aetiology.

Some congenital abnormalities (e.g. omphalocoele, complex heart disease) have known associated with chromosomal disorders. Invasive tests for conventional karyotype would be offered. This enables detection of aneuploidy, relatively large deletion and duplication (5-10 Mb) and other structural rearrangements such

as balanced and unbalanced translocations. If a chromosomal abnormality is present, the prognosis is poorer.

For many other structural anomalies, they might be related to single-gene disorders. In previous years, invasive tests may not be helpful because the genetic defect, if any, would be too small to be detected by conventional karyotype. Unless there is a family history and the affected members have been worked up, prenatal genetic diagnosis is often not possible.

As cytogenetic technology advances, aCGH makes detection of much smaller chromosomal imbalances possible, usually at a resolution of 10-400Kb. This brings revolutionary changes to prenatal diagnosis. A detailed foetal structural scan in the second trimester becomes the important 'key' in triaging high-risk pregnancies to invasive tests. Ultrasound detection of foetal anomalies depends on many factors -- type of anomalies, gestation, expertise of operators, protocols, length of scan time, the ultrasound machines and the patient's habitus. Including the 'extended views' to the checklist would definitely increase the yield.

In the new algorithm of prenatal diagnosis, when one or more foetal anomalies are detected on ultrasound, aneuploidy is still the first thing to look for. Conventional karyotype takes 2-3 weeks as it involves cell culture and chromosome processing to make them visible for microscopic evaluation. Rapid tests by quantitative fluorescent polymerase chain reaction (QF-PCR) are available for the common trisomies (i.e. trisomy 13, 18 and 21) and sex chromosome aneuploidy. Results are usually available within 2 days. After excluding common aneuploidies by a rapid test, aCGH could be the 'extension' test to consider while waiting for the full karyotype report. A large study showed that in the presence of foetal anomalies, non-common-benign CNV were detected in 8.1% after excluding aneuplodies. If multiple anomalies were present, the rate increased to 13.0%[10]. The detection rate varies with the organ system involved.

Knowing the genetic defect of congenital structural abnormalities helps the obstetrician to make a more accurate diagnosis and provides targeted information on the prognosis and recurrence risks to the parents. However, not all the foetal anomalies can find a 'genetic' diagnosis. One limitation of aCGH is that it could not detect genetic defects caused by point mutation. Occasionally, microarray may detect chromosomal changes known as 'variants of unknown significance' (VOUS) – the genetic changes and their association with disease are unknown. A very important point is, up to date, only a minority of affected children of congenital and developmental diseases receive a 'genetic' diagnosis.

Conclusion

In the new algorithm of prenatal diagnosis, NIPT and aCGH cannot work on their own; while conventional Down's screening and karyotype still preserve their roles. Ultrasound acts as the important 'road sign' diverting high-risk pregnancies to the more advanced tests. We believe this basic routine scan will no longer be 'sufficient' under this revolution – an 'extended' view of ultrasound would be required in future prenatal diagnosis.

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Financial Stability for Medical Professionals

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Dr Ares LEUNG

Securing a reliable professional indemnity scheme

Financial planning and protection is especially important to doctors in Hong Kong now. Recently, O&G is the first specialty forced unilaterally by our indemnity provider to have a huge conceptual change (though probably not necessarily financial) with indemnity that changes from occurrence to claim-made basis. Obstetricians are naturally worried about what to do when they retire, and such anxiety ripples off to affect everyone from rich seniors to new doctors considering which specialty to take. The lack of transparency in the operation of the current indemnity scheme adds to the uncertainty of our financial future and practices unless we take deliberate measures. While the status quo is no longer an option, the abrupt change of our professional indemnity arrangement has driven us to take the boldest and most forward-thinking strategy of engaging new insurance provider as a breakthrough to the monopolised service. This new strategy will set the course for attaining our need for increased transparency and certainty of our indemnity scheme, which may lend reference to other specialties soon. I am optimistic that we are leading to a more promising future.

Group practices should also take this opportunity to identify their need and choose the appropriate scheme of 'clinic professional indemnity' to cover adverse patient consequence from work of their staff. A clinic assistant of a 2-doctor clinic is staff of the clinic, not of a particular doctor. Professional indemnity of any doctor does not usually cover work of these clinic staff, even when one asks existing professional indemnity providers. Luckily, this type of group practice indemnity is affordable.

Planning your financial future

Now we move into some less studied aspects in personal asset management. We begin with an interesting fact. Americans keep us usually happy with investments: often up until we die! American assets, including stocks, are subject to huge estates tax in America. Therefore, when one is smiling about his wisdom on the choice of American stocks, he has to look after his own health.

Fixed assets are usually preferred by many Chinese for investment and wealth management. Property ownership is difficult to transfer even inside the family if apartments are under personal names. Although subject to tax and administrative hurdles with various administrations including that of HK, it is generally good planning to hold fixed assets with limited companies, which adds flexibility to financial planning.

Doctors do not usually attract adverse publicity in the lay media, up till the last decade. The society has changed and success/wealth could attract gossip. Present law allows tracing of local company directorship and property ownership down to the person. A doctor may therefore be subject to 'investigation' by tabloid media up to 'weighing of assets'. The exercise may be triggered by genuine scandals or mere curiosity related to fame. Privacy of ours and our loved ones is then at stake. It is very simple to hold assets with foreign companies. Accountants and lawyers are all too happy to advise us on logistics, and their implications on estate planning. However, an unwelcome consequence could be relative inflexibility with mortgages. One may need to consult a banker before leaping into action.

Financial consultants advise us to diversify our investment and assets, ranging from volatile (high fluctuation) to stable ones, high risk to low risk options in the form of stocks to bonds, precious metals to jewellery, single equities to unit trusts, exotic pieces of art to daily encounters like taxis. Any vehicle is a good one, as long as the investor studies it, likes it, and invests carefully & diligently. It may be noted that local assets tend to be rather volatile, probably in relation to our small but very open economy. Doctors are usually conservative and we may not like big swings. We may therefore consider part of our portfolio under non-local basis. Again, in-depth analysis and diligent follow-up are necessary. In addition, overleveraging is risky and may even affect our core work of doctoring. When emotions are not disturbed by assets, we own the asset and it works for us. If asset fluctuation disturbs our emotions badly, the condition reverses and we work for our money. Often a key to success is maintenance of cash enough to weather through a duration of adversity.

Regular deposits from cash into another asset class, such as stock or unit trust, is often described as a good way to accumulate wealth. It is important to examine penalty clauses with institutional financial plans like these. Often one has to finish the exercise over a promised duration of time, sometimes spanning over decades. Penalties may be calculated from total asset values if a 'premature end' is necessary. We all know how uncertain we are, about equity prices. We may encounter a financial crash at the time the plan finishes. However we are fined if we redeem it early! Strict inflexibility is not a good companion to investment.

Another problem with investment through unit trusts is the handling charges, usually up to several percent at purchase or redemption. If one sticks to an index fund for regular deposits, often the handling charges are not as high.

A major change over the past several decades is the way we leave riches to our loved ones, e.g., our children. We may want to help them financially jump start in life, especially when they raise their own family. The next generation unluckily also faces a very high divorce rate. We already have a tale of divorce in the child of a rich father. Sadly, a slice of the father-in-law's wealth was targeted during the unhappy separation. Readers may like to explore concepts with family trusts, so that the children are not immediate owners of estates. Trust costs may not be as expensive as it is commonly believed. Trusts are also widely used for asset protection in America where professional indemnity is often inadequate.

Last but not least, it is very important to invest in our own health with physical exercises. It is also very important to invest in relationship with our loved ones. Health and family worth way more than all other riches together.

Radiology Quiz

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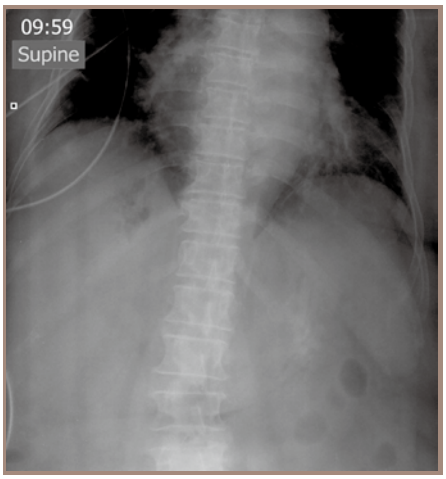

A 69-year-old lady with past history of poor DM control presented with high fever. Blood tests revealed deranged liver function tests.

Questions:

1. What are the findings on this AXR?
2. What further imaging modalities would be useful to confirm your suspicion?
3. What treatment would be needed?

(See P.36 for answers)

Date / Time	Function	Enquiry / Remarks
3 SAT 8:00 PM	**FMSHK Officers' Meeting** Organiser: The Federation of Medical Societies of Hong Kong; Venue: Gallop, 2/F, Hong Kong Jockey Club Club House, Shan Kwong Road, Happy Valley, Hong Kong	Ms. Nancy CHAN Tel: 2527 8898
4 SUN 1:00 PM	**HKMA Bridge Tournament 2015 (Professional IMP Pairs)** Organiser: The Hong Kong Medical Association; Chairman: Dr. LAM Hon Shing; Venue: Mariner's Club	Miss Denise KWOK Tel: 2527 8285
2:00 PM	**CPA Cup – National Day Celebration Dragon Boat Invitational Race 2015** Organiser: Hong Kong Institute of Certified Public Accountants; Chairman: Dr. YAM Chun Yin; Venue: Sai Kung	Mr. Ian KWA Tel: 2527 8285
5 MON 7:30 PM	**Melting kidneys: To drain or not to drain?** Organiser: Hong Kong Urological Association; Chairman: Dr. Chui Ka Lun; Speaker: Dr. Wong Hoi Fai Julius; Venue: Multi-disciplinary Simulation and Skills Centre, 4/F, Block F, QEH	Ms. Tammy Hung Tel: 9609 6064 1 CME Point
6 TUE 1:00 PM	**HKMA Kowloon West Community Network - Update on Non-Alcoholic Fatty Liver Disease (NAFLD)** Organiser: HKMA Kowloon West Community Network; Chairman: Dr. WONG Wai Hong, Bruce; Speaker: Dr. HSU Yau Que; Venue: Crystal Room IV-V, 3/F., Panda Hotel, 3 Tsuen Wah Street, Tsuen Wan, N.T.	Miss Hana YEUNG Tel: 2527 8285 1 CME Point
6:30 PM	**MPS Workshop – Mastering Professional Interactions** Organisers: The Hong Kong Medical Association & Medical Protection Society; Speaker: Dr. Lee Wai Hung, Danny; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F., Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points
8:00 PM	**HKMA Council Meeting** Organiser: The Hong Kong Medical Association; Chairman: Dr. SHIH Tai Cho, Louis; Venue: HKMA Wanchai Premises, 5/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Hong Kong	Ms. Christine WONG Tel: 2527 8285
8 THU 1:00 PM	**HKMA New Territories West Community Network - Certificate Course on Men's Health (Session 3): Helping the Man with Premature Ejaculation: Our Responsibility** Organiser: HKMA New Territories West Community Network; Chairman: Dr. CHAN Lam Fung, Lambert; Speaker: Dr. NG Wing Ying, Angela; Venue: Plentiful Delight Banquet (元朗喜尚嘉喜酒家), 1/F., Ho Shun Tai Building, 10 Sai Ching Street, Yuen Long	Miss Hana YEUNG Tel: 2527 8285 1 CME Point
1:00 PM	**HKMA Kowloon East Community Network - Update on Type 2 Diabetes Management in Elderly** Organiser: HKMA Kowloon East Community Network; Chairman: Dr. AU Ka Kui, Gary; Speaker: Dr. CHAN Chun Chung; Venue: Lei Garden Restaurant (利苑酒家) Shop no. L5-8, apm, Kwun Tong, No. 418 Kwun Tong Road, Kowloon	Miss Hana YEUNG Tel: 2527 8285 1 CME Point
1:00 PM	**HKMA Hong Kong East Community Network - Update on Diagnosis and Management of Psoriatic Arthritis** Organiser: HKMA Hong Kong East Community Network; Chairman: Dr. NGAN Sze Yuen, Silas; Speaker: Dr. CHAN Pak To; Venue: HKMA Wanchai Premises, 5/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Hong Kong	Ms. Candice TONG Tel: 2527 8285 1 CME Point
2:00 PM	**HKMA Structured CME Programme with Hong Kong Sanatorium & Hospital Year 2015 – The Contribution of Pathology to Personalized Medicine** Organiser: HKMA Kowloon East Organisers: The Hong Kong Medical Association & Hong Kong Sanatorium & Hospital; Speaker: Dr. Ma Shiu Kwan, Edmond; Venue: Function Room A, HKMA Dr. Li Shu Pui Professional Education Centre, 2/F, Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 1 CME Point
6:30 PM	**MPS Workshop – Mastering Difficult Interactions with Patients** Organisers: The Hong Kong Medical Association & Medical Protection Society; Speaker: Dr. Fung Shu Yan, Anthony; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F., Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points
10 SAT 2:15 PM	**CME Lecture - Refresher Course for Health Care Providers 2015/2016** Organiser: The Hong Kong Medical Association; Speaker: Dr. Wong Wai Yeung, Eddy; Venue: Training Room II, 1/F, OPD Block, Our Lady of Maryknoll Hospital, 118 Shatin Pass Road, Wong Tai Sin, Kowloon	Ms. Clara Tsang Tel: 2354 2440 2 CME Points
(11)	**5th Guangdong, Hong Kong and Macau (GHM) Sports Meet** Organiser: The Hong Kong Medical Association; Chairman: Dr. CHAN Hau Ngai, Kingsley / Dr. IP Wing Yuk; Venue: 廣州市大學城	Miss Ada SIU Mr. Ian KWA Miss Denise KWOK Tel: 2527 8285
11 SUN 11:30 AM	**RSCP Snooker Tournament 2015** Organiser: The Hong Kong Medical Association; Chairman: Dr. CHEUNG Wan Kit, Raymond; Venue: Youth Billiard Club	Miss Denise KWOK Tel: 2527 8285
13 TUE 6:00 PM	**1) Diagnosis and treatment of pulmonary hypertension in rheumatic diseases; 2) Case presentation** Organiser: The Hong Kong Society of Rheumatology; Chairman: Dr. KY Ying; Speaker: Dr. Tang Chun Pong; Venue: Hospital Authority Headquarters, Room 205S	Dr. Lee Ka Lai Tel: 9229 4616 1 CME Point
14 WED 7:30 AM	**Hong Kong Neurosurgical Society Monthly Academic Meeting – Can it not wait till tomorrow? Emergency treatment of ruptured aneurysms** Organiser: Hong Kong Neurosurgical Society; Chairman: Dr. Mak Hoi Kwan, Calvin; Speaker: Dr. Tsang Chun On, Anderson; Venue: M Block, Ground Floor, Lecture Theatre, QEH	Dr. Lee Wing Yan, Michael Tel: 2595 6456 1.5 CME Points
15 THU 6:30 PM	**MPS Workshop – Mastering Difficult Interactions with Patients** Organisers: The Hong Kong Medical Association & Medical Protection Society; Speaker: Dr. Fung Shu Yan, Anthony; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F., Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points
8:00 PM	**FMSHK Executive Committee Meeting** Organiser: The Federation of Medical Societies of Hong Kong, Veune: Council Chamber, 4/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Wanchai, Hong Kong	Ms. Nancy CHAN Tel: 2527 8898
17 SAT 1:30 PM	**KECN-HKCFP-UCH – CME Course for Health Personnel 2015 (Session 4) – Common Shoulder and Upper Limb Problems** Organisers: HKMA Kowloon East Community Network & Hong Kong College of Family Physicians & United Christian Hospital; Chairman: Dr. David CHAO; Speaker: Dr. LUK Man Sze, Karen; Venue: Lecture Theatre, G/F, Block P, United Christian Hospital, 130 Hip Wo Street, Kwun Tong, Kowloon	Miss Hana YEUNG Tel: 2527 8285 1 CME Point

Date / Time	Function	Enquiry / Remarks
17 SAT 2:30 PM	**MPS Workshop - Mastering Your Risk** Organisers: The Hong Kong Medical Association & Medical Protection Society; Speaker: Dr. Lee Wai Hung, Danny; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F., Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points
20 TUE 1:00 PM	**HKMA Kowloon West Community Network - Treatment and Prevention of Eczema Flares – by Combination Therapy (Latest AAD Guideline Update)** Organiser: HKMA Kowloon West Community Network; Chairman: Dr. LEUNG Kin Nin, Kenneth; Speaker: Dr. CHAN Kam Tim, Michael; Venue: Crystal Room IV-V, 3/F., Panda Hotel, 3 Tsuen Wah Street, Tsuen Wan, N.T.	Miss Hana YEUNG Tel: 2527 8285 1 CME Point
22 THU 1:00 PM	**HKMA New Territories West Community Network - Osteoporosis Management: A Practical Guide to Screening, Diagnosis and Treatment** Organiser: HKMA New Territories West Community Network; Chairman: Dr. TSANG Yat Fai; Speaker: Dr. YIP Wai Man; Venue: Pearl Ocean, 1/F., Gold Coast Yacht and Country Club, 1 Castle Peak Road, Castle Peak Bay, Hong Kong (黃金海岸鄉村俱樂部 - 遊艇會一樓金霞殿)	Miss Hana YEUNG Tel: 2527 8285 1 CME Point
8:00 PM	**FMSHK Foundation Meeting** Organiser: The Federation of Medical Societies of Hong Kong, Veune: Council Chamber, 4/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Wanchai, Hong Kong	Ms. Nancy CHAN Tel: 2527 8898
24 SAT 1:30 PM	**CME - Seminar on Infectious Diseases** Organiser: The Hong Kong Medical Association; Chairmen: Dr. CHOI Kin, Dr. SO Man Kit, Thomas and Dr. Lin Wai Chi, Ada; Speakers: Dr. Zee Sze Tsing, Jonpaul, Dr. Chan Man Chun, Jacky, Dr. Leo LUI and Dr. Wong Chun Kwan, Bonnie; Venue: Lecture theatre, 7/F, Block H, Princess Margaret Hospital, 2-10, Princess Margaret Hospital Road, Lai Chi Kok, Kowloon	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points
(25,26)	**The 4th Scientific Meeting of Asian Federation of Osteoporosis Societies cum the 16th Regional Osteoporosis Conference (AFOS 2015)** Organisers: Asian Federation of Osteoporosis Societies, The Osteoporosis of Macau and The Osteoporosis of Hong Kong; Chairmen: Dr. Wai Sin CHAN and Dr. Ka Kui LEE; Venue: Conrad Macao, Cotai Central	AFOS 2015 Macau Secreatriat Tel: (852) 2559 9973 CME Point (Pending) Website: www.afos 2015macau.org
28 WED 1:00 PM	**HKMA Central, Western & Southern Community Network - Doctor, What is this Swelling in My Neck?** Organisers: HKMA Central, Western & Southern Community Network; Chairman: Dr. LAW Yim Kwai; Speaker: Dr. WONG Chun Kuen; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F., Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	Miss Hana YEUNG Tel: 2527 8285 1 CME Point
29 THU 6:30 PM	**MPS Workshop - Mastering Shared Decision Making** Organisers: The Hong Kong Medical Association & Medical Protection Society; Speaker: Dr. Fung Shu Yan, Anthony; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F, Chinese Club Building, 21-22 Connaught Road, Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points
30 FRI 1:00 PM	**HKMA Yau Tsim Mong Community Network - Latest COPD Management – Dual Bronchodilation** Organiser: HKMA Yau Tsim Mong Community Network; Chairman: Dr. CHAN Wai Keung, Ricky; Speaker: Dr. WONG Ka Chun; Venue: Nathan Room, III-Hall, Level 1, Eaton, Hong Kong, 380 Nathan Road, Kowloon	Ms. Candice TONG Tel: 2527 8285 1 CME Point
31 SAT 2:30 PM	**MPS Workshop - Mastering Shared Decision Making** Organisers: The Hong Kong Medical Association & Medical Protection Society; Speaker: Dr. Fung Shu Yan, Anthony; Venue: HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F, Chinese Club Building, 21-22 Connaught Road, Central, Hong Kong	HKMA CME Dept. Tel: 2527 8452 2.5 CME Points

Upcoming Meeting

24/11/2015	**Integrative Management of Alopecia Areata & Hair Loss** Organiser: Association for Integrative Aesthetic Medicine, Hong Kong (AIAM); Speakers: Dr. Lam Pang and Prof. Fu Wen Shu; Venue: The Garden rooms, 2/F, Royal Garden Hotel, 69 Mody Road, TST East Tel: 3575 8600, Free for AIAM members; HK$50 for HKAIM members: HK$100 for non-members

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REFERENCE: 1. Muraro A et al. Pediatric Allergy and Immunology 2004; 15(3) : 196-205. 2. Szajewska H, Horvath A, Curr Med Res Opin. 2010;26(2):423-37. 3. Von Berg A, et al. J. Allergy Clin Immunol 2013. 4. Alexander D, Cabana M. JPGN 2010;50(4):422-30. 5. Høst A, Koletzko B, Dreborg S et al. Arch Dis Child. 1999;81(1):80-4. 6. Høst A, Halken S, Muraro A et al. Pediatr Allergy Immunol 2008:19(1):1-4. 7. Chouraqui JP, Dupont C, Bocquet A et al. Arch Pediatr 2008;15(4):431-42.

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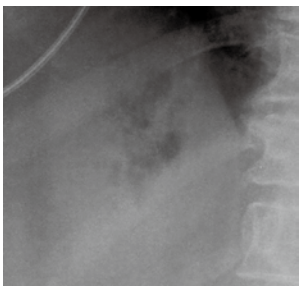
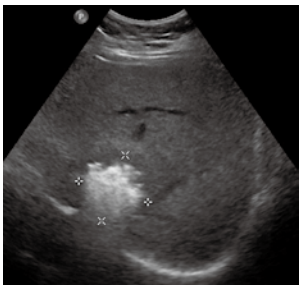
Answers to Radiology Quiz

Answer:

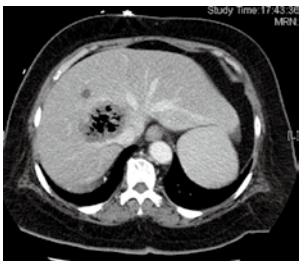
1. AXR: Abnormal cluster of gas densities is projected over liver shadow. This is suspicious of a gas-containing hepatic lesion, likely a liver abscess.



Magnified view of the RUQ.



2. Ultrasound or CT would be useful to confirm the diagnosis. Both studies were ordered by the referring clinican.
Ultrasound of liver confirmed the presence of a gas-containing lesion with strong echogenicities and dirty acoustic shadows. Features are compatible with a liver abscess.



Contrast CT abdomen accurately displayed the presence of a liver abscess.

3. Imaging-guided drainage would be required. CT-guided drainage was subsequently performed.

Discussion

Blood culture of the patient revealed Klebsiella pneumonia.
Endophthalmitis is a known association with klebsiella bacteraemia, and ophthalmological referral may be required.
Pyogenic liver abscess accounts for 80% of liver abscesses. Amoeba (Entamoeba histolytica) and fungi (Candida sp) account for the remaining 10% respectively.
Pyogenic liver abscesses are frequently polymicrobial. Causative organisms include E.coli (20.5%), Klebsiella pneumonia (16%), Bacteroides (11%) and Streptococcus milleri (12.2%).

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MBBS (HKU), FRCR
Resident, Department of Radiology, Queen Mary Hospital

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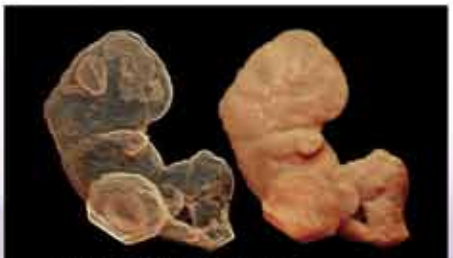

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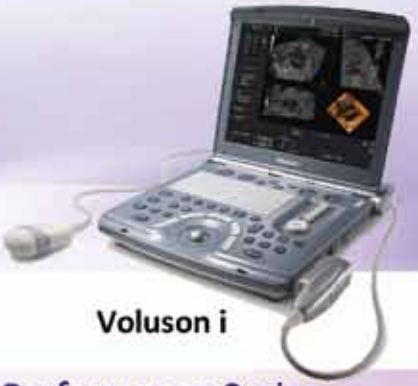

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