Impact of replacing or adding placental growth factor on Down syndrome screening: a prospective cohort study

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Abstract

Objectives: To assess whether adding placental growth factor (PlGF) or replacing pregnancy-associated plasma protein-A (PAPP-A) improves the first trimester combined test performance for trisomy 21. **Design:** Prospective observation Cohort Setting: The Chinese University of Hong Kong, China Sample: 11,518 women having a singleton pregnancy screened for trisomy 21 between December 2016 and December 2019 using the first trimester combined test. **Methods:** PlGF was prospectively measured and estimated term risk for trisomy 21 was calculated by 1) replacing PAPP-A with PlGF and 2) adding PlGF to the combined test which includes nuchal translucency, PAPP-A and free β-human chorionic gonadotropin (hCG). Main Outcome Measure: Screening performance, area under curve (AUC), detection rate (DR), screen positive rate (SPR) and false positive rate (FPR) **Results:** 29 women had trisomy 21. The combined tests DR, FPR and SPR were 89.7%, 5.7% and 6% respectively. DR when replacing PAPP-A or adding PlGF to the combined test remained unchanged. Replacing PAPP-A by PlGF increased FPR and SPR to 6.2% and 6.4% respectively. Adding PlGF to the combined test gave FPR and SPR rates of 5.5% and 5.7% respectively. Adding or replacing PlGF did not give a significant increase in AUC ($p>0.48$) over that of the combined test. **Conclusion:** Adding PlGF to the combined test or replacing PAPP-A with PlGF in the combined test did not improve trisomy 21 detection rate. Replacing PAPP-A by PlGF increased SPR, whilst adding PlGF resulted in only a marginal reduction in SPR.

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Running title:

PlGF for trisomy 21 screening

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Conclusion: Adding PlGF to the combined test or replacing PAPP-A with PlGF in the combined test did not improve trisomy 21 detection rate. Replacing PAPP-A by PlGF increased SPR, whilst adding PlGF resulted in only a marginal reduction in SPR.

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Reagents and equipment for the measurement of serum placental growth factor were provided free of charge by ThermoFisher Scientific.

Keywords Trisomy 21, Down syndrome, PlGF, PAPP-A, combined test, 1st trimester, screening, detection rate, false positive rate, screen positive rate

Tweetable Abstract: Adding PlGF does not increase combined Trisomy 21 test detection rate

Introduction

At present, the main role of placental growth factor (PlGF) in antenatal care has been in screening women for risk of preterm pre-eclampsia (PE) in the late first trimester or at later gestations. It is used on its own or in conjunction with soluble fms-like tyrosine kinase-1 (sFlt-1) as indicators for PE in asymptomatic women or in women who present with signs or symptoms of PE.11Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. N Engl J Med 2017;377:613-22 22Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med 2016;374):13-22 The finding that reduced levels of PlGF in maternal circulation is not only associated with PE but also with other adverse pregnancy and fetal outcomes such as being small for gestational age and aneuploidy has led to the discussion as to whether PlGF can fulfill a dual function. Specifically, the key research questions are whether PlGF can be used to screen for both PE and aneuploidy at 11-13 weeks of gestation and by replacing pregnancy associated plasma protein-A (PAPP-A) with PlGF the overall cost of screening is reduced. Alternatively, can PlGF be used as an additional biomarker as part of the first trimester combined test to increase the detection of trisomy 21.

There are similarities between PAPP-A and PlGF where both are produced by the placenta and are dependent on gestational age as well as maternal and pregnancy characteristics such as weight, smoking, mode of conception and ethnicity. However, whilst PAPP-A is shown to be consistently reduced in pregnancies affected by trisomies 13, 18 and 21, studies assessing the association between the level of PlGF and trisomy 21 have shown discordant findings. Maternal serum levels of PlGF have been reported as being both increased, reduced or no different in trisomy 21 affected pregnancies as compared to non-aneuploidy affected pregnancies.33Debieve F, Moiset A, Thomas K, Pampfer S, Hubinont, et al endothelial growth factor and placenta growth factor concentrations in Down's syndrome and control pregnancies. Mol Hum Reprod 2001;7:765-70 44Spencer K, Liao AW, Ong CY, L Geerts L, Nicolaides KH. First trimester maternal serum placenta growth factor (PIGF) concentrations in pregnancies with fetal trisomy 21 or trisomy 18. Prenat Diagn 2001;21:718-22 55Lambert-Messerlian GM, Canick JA. Placenta growth factor levels in second-trimester maternal serum in Down syndrome pregnancy and in the prediction of preeclampsia. Prenat Diagn 2004;24:876-80 66Zaragoza E, Akolekar R, Poon LCY, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11-13 weeks in chromosomally abnormal pregnancies. Ultrasound Obstet Gynecol 2009;33:382-6 Earlier studies have reported that the addition of PlGF to the conventional first trimester combined test of nuchal translucency (NT), free β-human chorionic gonadotropin (hCG) and PAPP-A has improved the detection rates of trisomy 21 by 1 to 2% for a given false positive rate. Possible reasons for the PlGF divergence include method used in PlGF measurement, ELISA or immunoassays, as well as underlying differences between assay PlGF isomer recovery and cross-reactivity. 77Cheng YKY, Poon LCY, Shennan A, Leung TY, Sahota DS. Intermanufacturer comparison of automated immunoassays for the measurement of soluble FMS-like tyrosine kinase-1 and placental growth factor. Pregnancy Hypertens 2019;17:165-71 Other factors which could have impacted on the earlier findings are PlGF stability due to length of time before processing and environmental temperatures in samples while being transported to central laboratories.88Cowans NJ, Alfthan H, Stenman UH, Spencer K. Stability of first trimester placental growth factor in serum and whole blood. Prenat Diagn 2011;31:1193-7 One factor common in many studies was that analysis was based on previously stored, as opposed to freshly acquired, serum samples with unknown number of freeze–thaw cycles and unknown length of storage time, if reported, and modelling of screening performance based on case-control findings.99Kagan KO, Hoopmann M, Abele H, Alkier R, Lüthgens K. First-trimester combined screening for trisomy 21 with different combinations of placental growth factor, free ß-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2012;40:530-5 Furthermore, samples in prolonged storage could be subject to freezer burn due to loss of water molecules resulting in increased measured concentrations.1010Blow, N. Biobanking: freezer burn. Nat Methods 2009:6; 173–178

The objective of this study was to determine whether incorporating PlGF as an additional or alternative marker for trisomy 21 screening would be clinically justifiable and of added benefit.

Materials and methods

Study population

This was a prospective cohort study of Chinese women with singleton pregnancies who attended for their Hong Kong Hospital Authority Universal Down Syndrome screening test at 11-13^{+6}weeks of gestation between December 2016 and December 2019 at the Prince of Wales Hospital, Hong Kong SAR.

Consented women underwent a structured ultrasound examination to document fetal viability, fetal NT thickness, fetal crown rump length (CRL) and absence of major fetal abnormalities. In all cases, maternal blood was drawn on the same day as the scan for determination of PAPP-A, free β-hCG and PlGF concentration levels using the BRAHMS KRYPTOR Compact Plus or Gold analysers (ThermoFisher Scientific, Hennigsdorf, Germany) at the Obstetrics Screening Laboratory of the Chinese University of Hong Kong. In addition, mean arterial pressure and uterine artery pulsatility index were documented as part of ongoing studies for risk assessment of preeclampsia.11Chaemsaithong P, Pooh RK, Zheng MM, Ma RM, Chaiyasit N, et al. Prospective evaluation of screening performance of first-trimester prediction models for preterm preeclampsia in an Asian population. Am J Obstet Gynecol 2019;221:650.e1-650.e16

Measured fetal NT, PAPP-A and free β-hCG were converted to their multiple of expected median (MoM) values using previously published expected median values in Chinese. 22Sahota DS, Leung TY, Fung TY, Chan LW, Law LW, et al. Medians and correction factors for biochemical and ultrasound markers in Chinese women undergoing first trimester screening for trisomy 21. Ultrasound Obstet Gynecol 2009;33:387-93 Gestational age at the time of screening was determined by CRL measurement using a previously published Chinese dating formula.33Sahota DS, Leung TY, Leung TN, Chan OK, Lau TK. Fetal crown-rump length and estimation of gestational age in an ethnic Chinese population. Ultrasound Obstet Gynecol 2009;33:157-60 Women were informed of their estimated term risk for trisomies 13, 18 and 21 based on their age, fetal NT, PAPP-A and free β-hCG MoM levels. Women with a term risk of 1:250 or higher were counselled and offered one of three options of fetal karyotyping after undergoing an invasive procedure (chorionic villous sampling or amniocentesis), seeking a diagnostic or non-invasive prenatal commercial test from a private specialist or opting for no further tests. Karyotype results were provided by our in-house prenatal diagnostic laboratory using either conventional karyotype or chromosomal microarray analysis.

Details on screening, follow-up management option selected, result of testing and pregnancy outcome were documented in our screening centre Laboratory Information System. Fetuses of screened pregnancies were considered to be phenotypically 'normal' at birth, if (1) the pregnancy was not reported as a false-negative case; (2) the fetus did not have any congenital abnormalities at birth; or (3) diagnostic test results in cases screened positive indicated that the pregnancy was euploidy (46XX/46XY) or had a karyotype considered to be a normal variant (balanced translocation, inherited maternal/paternal).

All scans were performed by midwives and doctors, accredited and annually recertified to assess the fetal NT by the Fetal Medicine Foundation (FMF, London, United Kingdom). Ultrasound providers, PAPP-A and free β-hCG MoMs were subject to internal quality assurance assessment for central tendency and dispersion using target plots.44Sahota DS, Chen M, Leung TY, et al. Assessment of sonographer nuchal translucency measurement performance—central tendency and dispersion. J Matern Fetal Neonatal Med 2011;24:812-6 Daily quality control (QC) samples with known low, intermediate and high concentrations were measured and monitored on both analysers to determine inter-and intra-day variation. In addition, the laboratory is a participant in two United Kingdom National External Quality Assurance Schemes (UKNEQAS), one for aneuploidy screening and second for quality assurance of PlGF measurements. The Screening Laboratory has previously reported a consistent detection rate of 90% for trisomy 21 affected pregnancies since it introduced the first trimester combined screening test in 2003 and that 5-6% of screened pregnancies are screened high risk.55Leung TY, Chan LW, Law LW, Sahota DS, Fung TY, et al First trimester combined screening for trisomy 21 in Hong Kong: outcome of the first 10,000 cases. J Matern Fetal Neonatal Med 2009;22:3004-, 66Sahota DS, Leung WC, To WKW, Lau ET, Leung TY. Prospective assessment of the Hong Kong Hospital Authority universal Down syndrome screening programme. Hong Kong Med J 2013;19:101-8 Women were not informed of their estimated risk for trisomy 21 based on PlGF.

Statistical analysis

PlGF concentration levels were converted to MoMs by performing a regression analysis to allow for change with gestational age, weight, smoking and method of conception (spontaneous or *in vitro*fertilization (IVF)) in the first 500 women screened. The PlGF MoM in these 500 women were then retrospectively calculated and recorded in our screening database. PlGF MoMs in subsequent screened pregnancies were prospectively calculated along with the estimated risks for trisomy 21 as described below.

Trisomy 21 risks using PlGF alone or in conjunction with other screening markers were determined using the multivariate Gaussian model approach currently used as a standard method in risk calculation software.11Reynolds TM, Penney MD. The mathematical basis of multivariate risk screening: with special reference to screening for Down's syndrome associated pregnancy. Ann Clin Biochem 1990;27:452-8 The expected PlGF MoM distributions in unaffected and trisomy 21 affected pregnancies were based on an earlier collaborative study. 22Han J, Liu H, Xu ZP, Cuckle H, Sahota D, et al. Maternal serum PlGF (placental growth factor) in Chinese women in the first trimester undergoing screening for Down syndrome. Eur J Obstet Gynecol Reprod Biol 2016;201:166-70 This study indicated that $\log_{10}$ PlGF MoM distribution

means and standard deviation in unaffected pregnancies were 0 and 0.1638, respectively, whilst corresponding figures in trisomy 21 affected pregnancies were 0.1979 and 0.1511.[17] The assumed correlation between $\log_{10}$ PlGF MoM and $\log_{10}$ PAPP-A MoM, $\log_{10}$ free β-hCG and $\log_{10}$ NT MoMs in unaffected pregnancies were 0.285, -0.019 and -0.027, respectively, whilst that in trisomy 21 affected pregnancies were respectively 0.0435, -0.121 and -0.337.[17] Modeling in our earlier study indicated a screening test based on maternal age, fetal NT, PAPP-A, free β-hCG and PlGF MoMs was expected to have a 96.2% detection rate for trisomy 21 for a 5% false positive rate. [17]

Three risks for trisomy 21 were prospectively estimated, one based on the conventional combined test, a second based on age, fetal NT, free β-hCG and PlGF and lastly one based on age, fetal NT, free β-hCG, PAPP-A and PlGF.

Area under the receiver operating characteristic (ROC) curve (AUC), detection rates and false positive rates were calculated for the combined risk as well as estimated risks based on replacement of PAPP-A by PlGF as well as the addition of PlGF to the conventional combined test. AUCs among the different first trimester combined tests were compared using the Delong test. 33DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44: 837-45 McNemar test was used to determine if replacement of PAPP-A by PlGF or the addition of PlGF resulted in a significant number of pregnancies being reallocated with regard to risk status for trisomy 21.

All trisomy 21 risks were estimated using our in house laboratory risk calculation software whilst all other analyses were performed using SPSS for Windows version 20 (SPSS, Illinois, USA) and MedCalc Statistical Software version 18.10.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Results

PlGF was measured in addition to the other trisomy 21 screening biomarkers in 11,518 women. 29 (0.25%) women were confirmed as having trisomy 21 affected pregnancy. The maternal, pregnancy and screening marker characteristics of these women are presented in Table 1. The detection rate of the first trimester combined test was 89.7% (95% confidence interval (CI) 72.6 to 97.8%) for a 5.7% (95%CI 5.3 to 6.2%) false positive rate.

The best fit regression model for PlGF was log-linear with gestational age in days, weight (kg) and smoking being significant independent predictors of its level. Expected median $\log_{10}$PlGF in unaffected pregnancies was estimated as being = 2.50220583

-0.03872797 * gestational age + 0.00032803 * gestational age^2 - 0.00146749 * weight + 0.07319080 * smoker (1 for smoker or 0 for non-smoker) (R^2=0.11).

The mean $\log_{10}$ PlGF MoM (standard deviation: SD) in unaffected and trisomy 21 affected pregnancies were 0.0000 (0.1939) and -0.2449 (0.2515), respectively. The correlations between $\log_{10}$ PlGF MoM, $\log_{10}$ free β-hCG MoM and $\log_{10}$ PAPP-A MoM in unaffected pregnancies were respectively 0.0783 (95% CI 0.0622 to 0.0944) and 0.3184 (95% CI 0.3037 to 0.3329) and statistically significant ($p<0.001$). The respective correlations in those with trisomy 21 were -0.2566 (95% CI -0.5600 to 0.1076) and -0.1331 (95% CI -0.4655 to 0.2322) and not statistically significant ($p>0.16$). The respective correlations in the pooled data were 0.0754 (95% CI 0.0593 to 0.0916) and 0.3205 (95% CI 0.3058 to 0.3349). The correlation between $\log_{10}$ PlGF MoM and gestational age at screening was not significant ($r=0.13$, $p=0.48$).

Figure 1 shows the ROC of PlGF based trisomy 21 screening tests compared to the current combined first trimester test. Delong test indicated that the difference in AUC between the combined test and after replacing PAPP-A or by adding PlGF as an additional marker was not significant AUC Combined vs Replacing PAPP-A: 0.984 (95% CI: 0.981 to 0.986) vs. 0.982 (95% CI: 0.980 to 0.985), p=0.58; Combined vs Adding PlGF: 0.984 (95% CI: 0.981 to 0.986) vs. 0.979 (95% CI: 0.976 to 0.981); p=0.36).

For a term risk cut-off of 1:250, the detection rate and false positive rate based on replacing PAPP-A by

PlGF in the combined test were 89.7% and 6.2%, respectively. The corresponding figures for the same threshold after adding PlGF to combined screening were 89.7% and 5.5%, respectively. Screening follow-up action remained unchanged in 96.0% and 97.8% of women had we reported trisomy 21 risk based on replacing PAPP-A by PlGF or adding PlGF to the existing combined test.

Post screening follow-up would have changed in 460 (4.0%) women by replacing PAPP-A by PlGF, with 52 (0.45%) women previously having a term risk of <1:250 now having a risk [?]1:250. When PlGF was added to the combined test post screening, follow-up would have changed in 257 (2.2%) women, with 29 (0.45%) women previously having a term risk of [?]1:250 now having a term risk of <1:250.

Discussion

Main findings

Our current prospective cohort study has confirmed the findings from our earlier case-control study that PlGF is reduced in trisomy 21 affected pregnancies. In addition, our study has shown that adding PlGF to the combined test does not increase the detection rate and that replacing PAPP-A with PlGF in the combined test has increased rather than reduced the screen positive rate. Our data would indicate that screening centres providing trisomy 21 screening and either concurrently performing or considering adding preeclampsia screening will need to measure both PAPP-A and PlGF and thus expect no reduction in laboratory reagent costs if seeking to maintain the existing screening test performance.

The strengths of our study were firstly its prospective design, secondly the use of both internal and external quality assurance assessment for all screening markers and thirdly its sample size, which allowed assessment of the impact on false positive and screen positive rates between the three risk estimation options. Although the number of trisomy 21 affected pregnancies was not high, they were sufficient in number to allow us to highlight that adding PlGF or replacing PAPP-A by PlGF did not improve the detection rate, especially if the screening centre is already achieving a high detection rate for the expected false positive and screen positive rates using the conventional combined test.

Interpretation

Our finding and conclusions are in agreement with a recent study by Maser Zumaeta and colleagues, which has concluded that PAPP-A is the preferred marker for trisomy 21 screening, and that replacing PAPP-A with PlGF increases the false positive and screen positive rates.11Mazer Zumaeta A, Wright A, Syngelaki A, Maritsa VA, Bardani E, et al. Screening for trisomies at 11-13 weeks' gestation: use of PAPP-A, PlGF or both. Ultrasound Obstet Gynecol 2020 doi: 10.1002/uog.22140 In a parallel study the same authors have reported that PlGF is the preferred biochemical marker for preeclampsia screening at 11-13 weeks of gestation, and that using PAPP-A instead of PlGF reduces preeclampsia screening sensitivity.22Mazer Zumaeta A, Wright A, Syngelaki A, Maritsa VA, Da Silva AB et al. Screening for pre-eclampsia at 11-13 weeks' gestation: use of pregnancy-associated plasma protein-A, placental growth factor or both. Ultrasound Obstet Gynecol 2020 doi: 10.1002/uog.22093. Our data and analysis indicate that previous studies reporting increased or similar levels of PlGF relative to unaffected pregnancies, as opposed to reduced levels of PlGF in trisomy 21, were probably due to the impact of case-selection, potential sample degradation or underlying differences between earlier PlGF assays and current assays. Our earlier study indicated that PlGF in stored samples remained stable for at least 3 years 33Law LW, Sahota DS, Chan LW, Chen M, Lau TK, et al. Effect of long-term storage on placental growth factor and fms-like tyrosine kinase 1 measurements in samples from pregnant women.J Matern Fetal Neonatal Med 2010;23:1475-80, however its stability when serum samples were stored for longer periods remained unknown. It has been shown that duration of storage time accounts for up to 35% of plasma protein concentration variation in frozen biobank samples of healthy women. 44Enroth S, Hallmans G, Grankvist K, Gyllensten U. Effects of Long-Term Storage Time and Original Sampling Month on Biobank Plasma Protein Concentrations. EBioMedicine 2016;12:309-14 Sensitivity of reported PlGF concentrations to specific PlGF isoforms has also been reported[7,] 55Nucci M, Poon LC, Demirdjian G, Darbouret B, Nicolaides KH. Maternal serum placental growth factor (PlGF) isoforms 1 and 2 at 11-13 weeks' gestation in normal and pathological pregnancies. Fetal Diagn Ther. 2014;36:106-16 Nucci

et al. reported that the PlGF-2 isoform of PlGF is more abundant than the PlGF-1 isoform throughout pregnancy,[24] whilst Cheng et al. reported that current assays had cross reactivity to PlGF-2 ranging from 10 to 21%, whilst PlGF-1 isomer recovery ranged from 38 to 60%.[7]

In the vast majority of cases adding PlGF or replacing PAPP-A with PlGF would not have changed whether women were screened high risk ([?]1:250) or low risk (<1:250) for trisomy 21. Women whose status did change, particularly when PlGF was added to the combined test, were those in whom PAPP-A and PlGF MoMs were discordant and with an atypical screening marker pattern for trisomy 21, either PAPP-A MoM was reduced (<1 MoM) and PlGF MoM was increased (>1 MoM) or PlGF MoM was reduced and PAPP-A MoM was increased. Of the 29 women having a trisomy 21 affected pregnancy, 24 were screened as high risk by the combined test, the combined test plus PlGF and after replacing PAPP-A by PlGF. Each test identified 2 of the 5 remaining trisomy 21 affected pregnancies, and hence there was effectively a zero sum gain. One alternative would be to use non-probability based pattern recognition such as machine learning based on multi-layer neural networks to see if such an approach would allow increased flexibility and recognition of atypical trisomy 21 screening marker patterns. 66Koivu A, Korpimäki T, Kivelä P, Pahikkala T, Sairanen M. Evaluation of machine learning algorithms for improved risk assessment for Down's syndrome. Comput Biol Med 2018;98:1-7

The current screening model for trisomy 21 is based on probability based pattern recognition, namely that trisomy 21 is associated with increasing maternal age, increased fetal NT and free β-hCG MoMs, reduced levels of PAPP-A and PlGF MoMs relative to unaffected fetuses. Our current and earlier study, as well as studies reporting reduced levels of PlGF in trisomy 21 affected pregnancies all indicated significant and strong correlations between PlGF and PAPP-MoMs. This is in contrast to other screening markers used to estimate risk for trisomy 21 as the inter-marker correlations are negligible, indicating that each marker is providing additional as opposed to effectively the same information when used to estimate risks. Inclusion of an additional biomarker which is significantly correlated with an existing marker is thus, as our study has demonstrated, unlikely to provide additional screening benefit. Replacing PAPP-A with PlGF or adding PlGF would only increase costs as the cost of the PlGF assay is higher than that of the PAPP-A assay.

Our study highlights also the difference in determining screening performance based on modelling versus that observed empirically when the test is used on a day to day basis. The former presumes collected data is in full agreement with the risk estimation model assumptions. Our earlier case-control study indicated that adding PlGF to the combined test would be expected to achieve a detection rate of 96% for the same 5% false positive rate based on modelling even though the population in our earlier study was 2 years younger than in our current study.[17] Modelling, whilst useful remains only as a guide, and as our data indicate, models reporting expected detection rates in future should incorporate a random percentage of cases having measurement discordance between included screening markers.

Conclusion

Adding PlGF or replacing PAPP-A with PlGF in the current first trimester combined test for trisomy 21 screening in screening centres performing both trisomy 21 and preeclampsia screening would not significantly improve the detection rate for trisomy 21 but would increase the screen positive rate if PAPP-A is replaced by PlGF or result in a small reduction in the screen positive and false positive rates if PlGF is added to the combined test.

Disclosures

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Reagents and equipment for the measurement of serum placental growth factor were provided free of charge by ThermoFisher Scientific that had no involvement in the study design; in the collection, analysis and

interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Contribution to authorship

DS Sahota had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. LC Poon and DS Sahota designed the study. P Chaemsaithong, YM Wah, SY Hui, YH Ting, KM Lam, TY Leung were responsible for data collection and confirmation. DS Sahota analyzed the data. WT Sin and DS Sahota were in charge of data interpretation and writing the manuscript raft. WT Sin, LC Poon and DS Sahota made substantial revisions to the manuscript.

Ethics approval

Approval for the study was obtained from the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC Ref. No. 2016.152) in Hong Kong and the Ethics Committee of each participating hospital in other regions.

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