Fetal Down syndrome screening models for developing countries; Part I: Performance of Maternal Serum Screening

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Abstract

**Background:** To identify the performance of fetal Down syndrome (DS) screening for developing countries.

**Methods:** A prospective study on MSS (maternal serum screening) with complete follow-ups (n = 41,924) was conducted in 32 network hospitals in the northern part of Thailand. Various models of MSS were tested for performance.

**Results:** MSS based on Caucasian reference range resulted in very high false positive rate (FPR; 13%) in our country, compared to the rate of 7.8% with our own (Thai) reference range, whereas the detection rate was comparable. As individual screening, C-S (contingent first trimester screening including PAPP-A, and free beta-hCG, classified as a) high risk [> 1:30], indicated for invasive diagnosis; b) intermediate risk [1:30–1500], indicated for STS; and c) low risk [≤ 1:1500], need no further tests.) was the most effective model (sensitivity 84.9%, FPR 7.7%) but nearly one-third needed the second trimester test (STS) because of intermediate results. Additionally, about one-third had their first visits in the second trimester and had no chance of FTS (first trimester screening). C-S plus STS had a sensitivity of 82.4% and FPR 8.1% whereas independent first and second trimester screening model (I-S) gave the sensitivity of 78.4% and FPR of 7.5% but was much more convenient and practical.

**Conclusion:** C-S plus STS was the most effective models while I-S model was also effective and may be better for developing countries because of its simplicity and feasibility.

**Keywords:** Down syndrome, Prenatal screening, Prenatal diagnosis, Cost-benefit, Developing country
were measured for biomarkers at the study center using the same laboratory (fully-automated immunoassay, using DELFIA® Xpress system; Perkin Elmer, Waltham, MA, USA) and standard assay screening kits of serum biomarkers (PAPP-A, AFP, beta-hCG, and uE3). All assays were performed in batches to eliminate inter-assay variations.

**Risk determination**

The risk category was based on the screening in real practice, 1) STS (second trimester screening) at 15–20 weeks for women going for their first visit in the second trimester, including AFP, free beta-hCG, and unconjugated estriol, using cut-off of 1:250 for high risk; and 2) Contingent first trimester screening (C-S) for women going for their first visit at 10–14 weeks, including PAPP-A, and free beta-hCG, classified as a) high risk (> 1:30), indicated for invasive diagnosis; b) intermediate risk (1:30–1500), indicated for STS; and c) low risk (< 1:1500), need no further tests. The high risk women were referred for invasive diagnoses (amniocentesis) at the study center. Risk determination of the screening was based on the Caucasian reference ranges (built-in). The gestational age was based on ultrasound biometry of the crown-rump length in the first trimester or biparietal diameter/head circumference in the first half of pregnancy.

**Follow-up of pregnancies**

All recruited women were followed-up for pregnancy complications such as abortion, preterm labor, intrauterine growth restriction, pregnancy-induced hypertension, antepartum hemorrhage, intrapartum and postpartum complications. Fetal loss related to diagnostic procedures was also evaluated for later use in cost-benefit analysis. All newborns were prospectively assessed by the neonatologists / pediatricians in the team of researchers. Neonatal chromosome study was performed only for the fetuses clinically suspected of chromosomal abnormalities after evaluation by the neonatologists. Diagnosis of fetal DS was based on chromosome studies by chorionic villous sampling, amniocentesis, or postnatal studies, while non-DS was based on chromosome studies or the conclusion by the neonatologists in cases of absence of chromosome study results.

**Definition of primary screening models**

In real practice, the patients were managed using the screening based on Caucasian reference ranges (CRR) as mentioned above. However, the data permitted us to re-categorize the primary screening into several models, using Thai reference ranges (TRR) [14, 15], as follows: 1) Maternal age alone: High risk if maternal age is up to 35 years or more; 2) STS (second trimester screening) alone: The risk derived from serum levels of AFP, free b-
hCG and uE3; 3) FTS (first trimester screening): The risk derived from serum levels of PAPP-A and free b-hCG; 4) Contingent FTS screening (C-S): The risk derived from serum levels of PAPP-A and hCG) and categorized into three groups according to the risk as mentioned above; 5) C-S plus STS: Contingent FTS for women with first visit in the first trimester and STS alone for women with first visit in the second trimester; 6) Independent FTS and STS (I-S): FTS alone for women with first visit in the first trimester and STS alone for women with first visit in the second trimester.

Statistical analysis
The diagnostic performance (detection rate and false positive rate) was assessed for the various models mentioned above. Sample size estimation was based on previous studies [16–19], which reported that MSS had a sensitivity of ≥70% at a false positive rate of 5% for fetal DS screening among unselected pregnant women. Given a confidence level of 95% and acceptable error in diagnosis of 0.1, the project needed at least 72 fetuses with DS. The prevalence of fetal DS is about 1:600 at gestational age of 16 weeks. Therefore, the study needed a sample size of at least 43,200 tests. Statistical analysis was performed using IBM SPSS version 21.0(IBM SPSS Statistics for Windows, Released 2012. Armonk, NY: IBM Corp).

Results
Of 45,220 eligible pregnancies, 43,216 attended antenatal care clinics and met the inclusion criteria. Of them, 41,924 women accepted MSS (C-S plus STS based on CRR) either in the first trimester contingent screening or second trimester and complete data of final outcomes as shown in Fig. 1. Of all, 5405 (12.9%) were categorized as high risk (HR), including 4997 (92.45%) undergoing amniocentesis and 408 (7.55%) doing nothing. Of all, 74

![Fig. 1 Flow chart of screening cascades: contingent FTS plus STS based on Caucasian reference ranges with ethnic (Asian) factor correction](image-url)
pregnancies had fetal DS, including 61 and 13 with and without prenatal detection, respectively. The prevalence of fetal DS was 0.18% or 1:567. Of non-affected cases, spontaneous fetal loss after 16 weeks was 59/36,927 (0.16%) and fetal loss rate among women undergoing amniocentesis was 33/4997 (0.66%). (Very high acceptability was observed in all steps, due to free of charge in this project).

About two-thirds of women (29,692; 70.8%) first attended antenatal care in the first trimester and had a chance of FTS and C-S whereas the remaining nearly one-third (12,232; 29.2%) had first visits in the second trimester and could undergo only STS.

Screening performance of the various models is shown in Table 1. Of note, screening (C-S plus STS) based on the CRR has the comparable detection rate when compared to that based on TRR (86.8% vs 84.9%; 76.2% vs 76.2 and 83.8% vs 82.4% for C-S, STS and C-S plus STS, respectively). However, the false positive rate was much higher with the CRR when compared to that based on TRR (13.7% vs 7.7%; 11.0% vs 9.2 and 12.9% vs 8.1% for C-S, STS and C-S plus STS, respectively).

Of all screening tests using TRR, FTS had better screening performance than STS (detection rate of 79.2% vs 76.2%, and false positive rate of 6.8% vs 9.2%, respectively). Nevertheless, C-S had a significantly higher detection rate when compared with simple FTS (84.9% vs 79.2%; Chi-square test; p-value < 0.001); with slightly higher false positive rate (7.7% vs 6.8%; Chi-square test; p-value < 0.001).

Among C-S, the actual risk based on CRR was classified as low risk (LR), intermediate (IR) and high risk

### Table 1
Summary of the primary screening performance for fetal Down syndrome

| Final Risk  | N (%)  | Down Syndrome | Sensitivity | False Positive |
|-------------|--------|---------------|-------------|----------------|
| **Maternal Serum Screening Based on Caucasian Reference Ranges** |
| Contingent screen (C-S) | LR | 25,580 (86.2%) | 7 | 86.8% | 13.7% |
|  | HR | 4112 (13.8%) | 46 |  |
|  | Total | 29,692 (100%) | 53 |  |
| Second trimester serum screen (STS) alone | LR | 10,877 (88.9%) | 5 | 76.2% | 11.0% |
|  | HR | 1355 (11.1%) | 16 |  |
|  | Total | 12,232 (100.0%) | 21 |  |
| C-S plus STS | LR | 36,457 (87.0%) | 12 | 83.8% | 12.9% |
|  | HR | 5467 (13.0%) | 62 |  |
|  | Total | 41,924 (100%) | 74 |  |
| **Maternal Serum Screening Based on Thai Reference Ranges** |
| Contingent screen (C-S) | LR | 27,361 (92.1%) | 8 | 84.9% | 7.7% |
|  | HR | 2331 (7.9%) | 45 |  |
|  | Total | 29,692 (100%) | 53 |  |
| Second trimester serum screen (STS) alone | LR | 11,093 (90.7%) | 5 | 76.2% | 9.2% |
|  | HR | 1139 (9.3%) | 16 |  |
|  | Total | 12,232 (100%) | 21 |  |
| C-S plus STS | LR | 38,454 (91.7%) | 13 | 82.4% | 8.1% |
|  | HR | 3470 (8.3%) | 61 |  |
|  | Total | 41,924 (100%) | 74 |  |
| First trimester serum screen (FTS) alone | LR | 27,627 (93.0%) | 11 | 79.2% | 6.8% |
|  | HR | 2065 (7.0%) | 42 |  |
|  | Total | 29,692 (100%) | 53 |  |
| I-S (Independently combined first and second trimester) | LR | 38,720 (92.4%) | 16 | 78.4% | 7.5% |
|  | HR | 3204 (7.6%) | 58 |  |
|  | Total | 41,924 (100%) | 74 |  |
| **Age-based Screening** |
| Combined First and Second Trimester | LR | 36,967 (88.2%) | 52 | 29.7% | 11.8% |
|  | HR | 4957 (11.8%) | 22 |  |
|  | Total | 41,924 (100%) | 74 |  |
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was the most effective serum screening
model (detection rate of 84.9% with false positive rate of
). c) In real practice, C-S plus STS gave the best
screening performance (detection rate of 82.4% with
false positive rate of 8.1%), d) Age-based screening had
the lowest detection rate (29.7%) and relatively high false
positive rate (11.8%).

In summary, a) Maternal serum screening (without
NT) based on CRR had a very high false positive rate
when compared to that based on TRR, b) As an individu-
al test, C-S was the most effective serum screening
model (detection rate of 84.9% with false positive rate of
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Discussion
The important insights gained from this study are: 1) CRR for maternal serum screening used in other parts of
the world may probably lead to erroneously high false
positive rate, resulting in excessive burden of amniocen-
tesis and unnecessary fetal losses. 2) Theoretically, C-S
was the most effective serum screening test. 3) In real
practice, C-S plus STS gave the best screening perfor-
ance since not all women were able to undergo FTS,
nearly one-third having first visits after first trimester.
Nevertheless, I-S is a more practical model in terms of
patient’s convenience of first visit timing and only once
screening.

Interestingly, two very unusual findings were observed
in MSS using CRR: 1) very high rate of intermediate risk
of FTS, more than 30% (but 20% when using Thai refer-
ence range), and 2) high false positive rate in both FTS
and STS. Because of the unacceptably high false positive
rate and intermediate risk rate of MSS using CRR, we
strongly recommended reference ranges of its popula-
tion instead of ethnic correction factor unless it has been
proven to be accurate in large sample size in its own
population. Importantly, the very high rate of amniocen-
tesis secondary to false positive rate is not only associ-
ated with the great number of fetal losses but the
burden of chromosome laboratories is also too problem-
atic for the government to include DS screening in
health coverage as a national policy. Finally, the per-
formance derived from our own reference range would
be better used to base cost-benefit analysis (CBA) on
subsequently. Cost-benefit directly depends on the per-
formance of the screening test, both sensitivity and spe-
cificity. The accuracy of diagnostic performance is very
important for further evaluation of CBA. The sensitivity
and specificity of the screening test must be based on
the real practice. It directly determines the number of
amniocenteses and non-invasive prenatal tests (NIPT) or
cell-free fetal DNA technique.

Considering the best model for developing countries,
several aspects must be taken into account: feasibility,
expertise requirement, simplicity, costs of screening tests
and invasive diagnosis, capacity in chromosome lab de-
velopment etc. Note that this study did not include inte-
grated tests, because of the high costs of double
screenings with small additional detection rate. It also
excluded NT and genetic sonogram, because of the need
for high expertise, not practical for extensive use in low
resource settings. FTS alone was not suitable since many
women had their first visit in late gestation. C-S plus
STS was most effective but had higher costs due to the
high rate of intermediate risk requiring STS and was
complicated by counseling as well as anxiety during
waiting for the final risk. Therefore I-S seems to be more
attractive, though with slightly lower detection rate.

Strength
The strengths of this study are as follows: 1) It is a pros-
pective large-scale population-based study. 2) All
models were based on feasibility and simplicity. 3) All
newborns, either high risk or low risk determined by
MSS, were evaluated for DS by pediatricians in the pro-
ject. 4) All samples were properly collected and trans-
ported and run at the same laboratories. We were
conscious of the logistics and temperature, which have
been proven to have an obvious influence on the serum
marker levels, as suggested by our preliminary study
[20], 5) The high homogeneity of the participants (Thai
ethnicity). 6) This project was undertaken under the
support of a non-profit organization without conflict of
interest.

Weakness
The weaknesses of this study are as follows: 1) Some
other well-known strategies like integrated screening or
fully combined first trimester screening were not in-
cluded; however, such strategies are not suitable for low
resource settings. 2) Trisomy13 and 18 were not taken
into account since they were not a major problem in de-
veloping countries and were considered incompatible
with life. 3) The uptake rate of MSS in this study could
not represent the real practice since all women in this

(HR); 18,336 (61.8%), 10,962 (36.9%) and 394 (1.3%), re-
respectively. However, when reclassified using TRR, LR, IR
and HR were: 23,314 (78.5%), 5984 (20.2%), and 394
(1.3%), respectively. Note that all cases classified as LR
and HR by CRR were still classified as LR and HR by
TRR but, importantly, more than one-third of IR by
CRR were reclassified as LR when using TRR. Interest-
ingly, with TRR the screening performance was much
better as indicated by a marked decrease in false positive
rate (13.7% vs 7.7%) while the detection rate was nearly
the same. Likewise, STS based on CRR had also a signifi-
cantly higher false positive rate than STS based on TRR
with the same detection rate (McNemar Chi-square test;
p-value < 0.001) as shown in Table 1. Age-based screen-
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tively high false positive rate (11.8%).

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resource settings. 2) Trisomy13 and 18 were not taken
into account since they were not a major problem in de-
veloping countries and were considered incompatible
with life. 3) The uptake rate of MSS in this study could
not represent the real practice since all women in this
project were offered the MSS free of charge. 4) The dataset originally used for categorization based on CRR could not be exactly the same as that based on TRR since the cases categorized as low risk and high risk by CRR in the first trimester did not contribute data for the second trimester screening, whereas the intermediate risk women did. In principle, if they were firstly categorized using TRR, those women might have become intermediate risk with contributing data for second trimester screening. 5) The models in this study were primarily focused on our national health care. Thus, the results might not be perfectly accurate for other countries’ strategies. However, we believe that this could probably be a model for several developing countries especially many parts of Asia.

Conclusion
MSS reference ranges derived from Caucasian pregnant women could not be effectively used with Southeast Asian women even with the use of racial factor correction. This is because the false positive rate is far too high. While the detection rate is comparable, the rate of amniocentesis (false positive) is high, leading to an increased burden of amniocentesis and chromosome laboratories as well as high fetal loss rate secondary to the procedure. Each geographical area should have its own reference ranges for its own population.

Abbreviations
AFP: Alpha-fetoprotein; CBA: cost-benefit analysis; CRR: Caucasian reference ranges; C-S: contingent first trimester screening; DS: Down syndrome screening; FPR: false positive rate; FTS: First trimester screening; hCG: human chorionic gonadotropin; HR: High risk; I-S: independent first and second trimester screening; LR: Low risk; MSS: Maternal serum screening; NIPD: non-invasive prenatal test; NT: Nuchal translucency; PAPP-A: Pregnancy-associated plasma protein A; STS: Second trimester screening; TRR: Thai reference range; uE3: unconjugated estriol

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Authors’ contributions
CW: contributed to the conception, design of the research and administration of the project, and drafting/revising the manuscript; SS: contributed to the conception, acquisition of data and administration of the project, and revising the manuscript; WP: contributed to acquisition of data, and revising the manuscript; FT: contributed to acquisition of data, and revising the manuscript; KS: contributed to acquisition of data, and revising the manuscript; SL: contributed to acquisition of data, drafting and revising the manuscript; KT: contributed to acquisition of data, and revising the manuscript; PJ: contributed to acquisition of data, and revising the manuscript; TT: contributed to the conception, design of the research, analysis or interpretation and drafting/revising the manuscript; All authors contributed to the interpretation and writing of the paper and approved the final version.

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Availability of data and materials
The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate
This study received ethical approval from the institute review boards of Faculty of Medicine, Chiang Mai University (Ethics Committee 4; Research ID 4981). All participants signed Informed Consent Forms.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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