Efficacy of WHO recommendation for continued breastfeeding and maternal cART for prevention of perinatal and postnatal HIV transmission in Zambia

Mary S Ngoma1,*,1, Amita Misir*2, Wilbroad Mutale3, Emmanuoloi Rampakakis4,5, John S Sampalis4,5, Angela Elong6, Sam Chisele7, Abel Mwale8, Jonathan K Mwansa9, Scholastica Mumba10, Mula Chandwe1, Richard Pilon11, Paul Sandstrom13, Samantha Wu12, Kristen Yee13 and Michael S Silverman6,14

Abstract

Introduction: To prevent mother-to-child transmission (MTCT) of HIV in developing countries, new World Health Organization (WHO) guidelines recommend maternal combination antiretroviral therapy (cART) during pregnancy, throughout breastfeeding for 1 year and then cessation of breastfeeding (COB). The efficacy of this approach during the first six months of exclusive breastfeeding has been demonstrated, but the efficacy of this approach beyond six months is not well documented.

Methods: A prospective observational cohort study of 279 HIV-positive mothers was started on zidovudine/3TC and lopinavir/ritonavir tablets between 14 and 30 weeks gestation and continued indefinitely thereafter. Women were encouraged to exclusively breastfeed for six months, complementary feed for the next six months and then cease breastfeeding between 12 and 13 months. Infants were followed for transmission to 18 months and for survival to 24 months. Text message reminders and stipends for food and transport were utilized to encourage adherence and follow-up.

Results: Total MTCT was 9 of 219 live born infants (4.1%; confidence interval (CI) 2.2–7.6%). All breastfeeding transmissions that could be timed (5/5) occurred after six months of age. All mothers who transmitted after six months had a six-month plasma viral load >1,000 copies/ml (p < 0.001). Poor adherence to cART as noted by missed dispensary visits was associated with transmission (p = 0.04). Infant mortality was lower after six months of age than during the first six months of life (p = 0.02). The cumulative rate of infant HIV infection or death at 18 months was 29/226 (12.8% 95 CI: 7.5–20.8%).

Conclusions: Maternal cART may limit MTCT of HIV to the UNAIDS target of <5% for eradication of paediatric HIV within the context of a clinical study, but poor adherence to cART and follow-up can limit the benefit. Continued breastfeeding can prevent the rise in infant mortality after six months seen in previous studies, which encouraged early COB.

Keywords: breastfeeding transmission of HIV; vertical transmission; option B+; infant survival; efficacy of WHO guidelines; perinatal transmission of HIV.

To access the supplementary material to this article please see Supplementary Files under Article Tools online.
recommended during pregnancy and postpartum breastfeeding to prevent MTCT (PMTCT). For women whose CD4 cells are <500 cells/µl, on-going maternal cART after COB is recommended to protect the mothers health [15]. For women whose CD4 >500 cells/µl, the WHO recommends either option B where maternal cART is discontinued after COB or Option B+ where maternal cART is continued indefinitely [15]. Unfortunately, there are only limited data on the impact of maternal cART on infant adverse events and PMTCT while breastfeeding beyond six months of age. Therefore, the quality of the supporting evidence for the new WHO guidelines is still noted as low to moderate [2,14,15]. We, therefore, carried out a study to assess the efficacy and safety of continued breastfeeding while the mother receives cART.

Methods
This was a prospective observational cohort study. Recruitment took place from December 2008 to December 2009 at the Chelstone Public Clinic in Lusaka, Zambia.

Inclusion criteria were: age ≥15 years, HIV seropositivity, confirmed pregnancy with the ability to initiate cART at 14–30 weeks gestation, intention to EBF for six months, ability to give informed consent and to attend follow-up visits. Exclusion criteria were previous cART (other than single-dose nevirapine or ≤1 month of zidovudine for PMTCT prophylaxis, which were not contraindications), known major illness likely to influence pregnancy outcome or place the participant at increased risk for adverse events from cART, including diabetes, severe renal, liver or heart disease, active tuberculosis, severe anaemia (Hgb <8 g/dl) or continuing therapy with medications, which are contraindicated for co-administration with ritonavir/lopinavir.

All mothers received zidovudine (ZDV) 300 mg/ lamivudine (3TC) 150 mg (Combivir®, ViiV healthcare, Middlesex, UK) bid and lopinavir 200 mg/ritonavir 50 mg (trade name Aluvia†, ViiV healthcare, Middlesex, UK) bid (including formula) and/or semi-solid/solid foods before six months.

Completion feeding (CF): Continued breastfeeding with the addition of solid or semi-solid foods beyond six months.

Replacement feeding: No breast milk at all, fed only non-human milk or semi-solids/solids.

Ethical approval for the study was obtained from the research ethics boards of the University of Zambia in Lusaka and also Lakeridge Health Corporation in Canada. All mothers provided written informed consent. The study funders had no role in the design of the protocol, conduct of the study, the analysis of data or the writing of the manuscript.

Statistical methods
The primary outcome of interest was the rate of HIV-free survival after an extended period (i.e. 12 months) of breastfeeding followed by gradual COB. Secondary outcomes of interest included HIV transmission rates and mortality particularly as they corresponded to the different time periods of exclusive breastfeeding (0–6 months), CF (6–12 months) and COB (12–18 months). The incidence of HIV transmission and death was described with the corresponding incidence rates up to 18 months post-partum for the above time periods. Denominators for these incidence rates include only those infants who remained at risk for transmission (i.e. were known to be HIV-uninfected and surviving) at the beginning of each relevant time period.

For all analyses, patients who were lost to follow-up were not included in the analysis for the periods when they were not available. This was decided in order to prevent these patients from being inadvertently considered as non-transmitting or surviving and thus artificially inflating efficacy. Categorical variables were summarized using frequency distributions and percentages, whereas continuous variables were described with the mean and standard deviation. We used independent samples t-test to assess statistical differences in (i) maternal age, maternal CD4 at birth and infant GA at birth between women lost to follow-up post-partum and those completing 24 months of follow-up and (ii) mean viral load between transmitting and non-transmitting mothers. We used Pearson’s chi-square test to assess statistical differences in (i) the viral load categories at six months between transmitting and non-transmitting mothers;
(ii) infant mortality based on presence or absence of low birthweight and (iii) HIV transmission between women missing vs. not missing a dispensary visit. Changes in viral load and viral load detection over six months of treatment were assessed with the paired t-test and the McNemar test, respectively. The incidence of HIV transmission and death was described with the incidence rate and the incidence rate ratio and the corresponding 95% confidence intervals (95% CI). A comparison of different time intervals with respect to these outcomes was performed using the person-time chi-square (χ²) test [21,22].

Predictors of adverse event count by time interval were investigated using a saturated Poisson regression model where time interval and individual person follow-up time were incorporated as adjusting covariates. Given that this was a saturated model, goodness of fit was not assessed. Over dispersion was examined by dividing the Pearson statistic by its degrees of freedom and was corrected, as required, by inflating accordingly the covariance matrix.

All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC), and all analyses were performed using a 0.05 significance level.

Results

Study population
Clinical baseline characteristics of the cohort are presented in Table 1.

Transmission
Transmission status at follow-up is presented in Figure 1. Overall, when considering all 231 live births, HIV status was known at 18 months or at the last scheduled test interval before their death (whichever came first) for 201/231 (87.0%). There was no difference between the women who were lost to follow-up postpartum and those who completed 24 months of follow-up when compared by maternal age (p = 0.55), maternal CD4 at birth (p = 0.89) or infant GA at birth (p = 0.946). A total of 9/219 (4.1%; 95% CI: 2.2–7.6%) infants acquired HIV during the study period. There was a trend towards a higher transmission rate/100 person-weeks during 6–12 months (0.0529/100 person-weeks) when compared to six weeks to six months (0/100 person-weeks; p = 0.167).

Details of the individual transmissions are found in Supplementary Table 2. Baseline maternal CD4 count (< 200 vs. > 200; odds ratio, OR < 350 vs. > 350, OR < 500 vs. > 500) was not correlated with infant mortality or postpartum transmission (data not shown).

Treatment failure and late HIV transmission
Five of five women who transmitted after six months had a plasma viral load (VL) > 1,000 at six months (range 3,252–459,866; mean 4.75 log₁₀ SD 0.82). In comparison, mean viral loads at six months in the 96 non-transmitting mothers tested were lower (mean 0.61 log₁₀ SD 1.4; p = 0.0001). Twelve of 96 (12.5%) non-transmitting mothers had a VL > 1,000 (p = 0.001) and 16/96 (16.7%) had a VL > 50 at six months (p = 0.001). Including all transmitting and non-transmitting mothers, more mothers had a detectable VL (> 50) at birth (40 of 99 (40.4%) than at six months (p = 0.002), and mean Log₁₀ viral load at six months (1.21, SD 1.94) was not higher than at birth (1.25, SD 1.73; p = 0.86). Data available on seven of nine transmissions showed one transmission (antepartum) to be associated with resistance to the cART regimen (K65R in mother and infant; Supplementary Table 3).

Mortality
Four of 279 (1.4%) mothers died including one maternal death with accompanying miscarriage/foetal death. Details of individual infant and maternal deaths are given in Supplementary Tables 4 and 5.

Two hundred and eleven of 231 (91.3%) live born infants had 24-month survival data, the remainder being lost to follow-up. Cumulative postnatal mortality was 25/231 live births (10.8%; 95% CI: 6.8–14.8%). Data on cumulative rate of HIV infection and/or death were available for 226 infants with a cumulative rate of 29/226 (12.8%; 95% CI: 7.5–20.8%) (includes 20 deaths of HIV-uninfected infants, 5 deaths in HIV-infected infants and 4 transmissions in surviving infants).

HIV-uninfected infant death rate was highest in the first six months of life, falling during the second six months with no evidence of any rise after COB at 1 year (Table 2).

Using the same intervals as in the recent BAN study, which showed an increase in HIV-uninfected infant mortality with COB at 28 weeks [9], HIV-uninfected infant mortality in our cohort fell from 0.2183/100 person-weeks (95% CI: 0.1162–0.626) during the first 28 weeks of life to 0.0755/100 person-weeks (95% CI: 0.0156–0.2016) during weeks 29–48 (incidence rate ratio = 0.346 (95% CI: 0.0983–1.22); p = 0.04).

Total mortality (HIV-infected and -uninfected infants) was also higher in the first six months of life 0.2839/100 person-weeks (95% CI: 0.1589–0.4564) than between 6 and 12 months 0.0813/100 person-weeks (95% CI: 0.022–0.1933; incidence rate ratio = 0.286 (95% CI: 0.069, 0.8989) (p = 0.02)).

Maternal adherence
Self-reported adherence to breastfeeding recommendations was requested at each visit with full data to COB or death being available on 205/231 infants (88.7%). Adherence was high with 92.8% EBF until six months and 79% CF thereafter. Only 4.5% had ceased breastfeeding completely at 6 months

Table 1. Maternal baseline characteristics (live born infants only)

<table>
<thead>
<tr>
<th>Maternal baseline characteristics</th>
<th>Available n</th>
<th>(N = 231)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, years (mean (SD))</td>
<td>226</td>
<td>28.1 (5.5)</td>
</tr>
<tr>
<td>Gestational age (GA) at birth, weeks, (mean (SD))</td>
<td>229</td>
<td>38 (3.5)</td>
</tr>
<tr>
<td>Sexually transmitted infections (STIs), n (%)</td>
<td>225</td>
<td>41 (18%)</td>
</tr>
<tr>
<td>Multiple birth, n (%)</td>
<td>225</td>
<td>7 (3%)</td>
</tr>
<tr>
<td>CD4 &lt; 350 at study entry, n (%)</td>
<td>209</td>
<td>125 (60%)</td>
</tr>
<tr>
<td>CD4 &lt; 200 at study entry, n (%)</td>
<td>209</td>
<td>45 (22%)</td>
</tr>
<tr>
<td>CD4 count, mean (SD)</td>
<td>209</td>
<td>353.8 (207.1)</td>
</tr>
<tr>
<td>Anaemia (haemoglobin &lt; 10 g/dl), n (%)</td>
<td>225</td>
<td>55 (24%)</td>
</tr>
</tbody>
</table>

Note: Detailed socio-demographic data are available in Supplementary Table 1.
and 97.8% had ceased breastfeeding by 15 months (Figure 2) (age range of COB 1.5–17 months).

Self-reported adherence to taking cART was unreliable as it correlated poorly with attendance at dispensary visits (data not shown). Thirty-six of 226 women (15.9%) missed at least one dispensary visit (i.e. were greater than two weeks late for a scheduled visit and therefore would have had a drug holiday due to medication shortage). Four of 35 (11.4%) women who missed a visit vs. 5 of 184 (2.7%) women who did not miss a visit transmitted HIV (p = 0.038).

Maternal and infant adverse events

In order to compare adverse events during different time periods, HIV-infected and HIV-uninfected infants are analyzed..
separately as in previous studies [9]. In HIV-uninfected infants (Table 3), FTT was greatest in the first six months of life (p < 0.001), the rates of diarrhoea rose after six months of age (during the period of CF) when compared with the first six months of life (EBF; p < 0.001) and the rates of respiratory tract infections rose during the period of COB (12–18 months of age) when compared with the periods of EBF (0–6 months) and CF (6–12 months; p < 0.001 for both comparisons). The number of HIV-infected infants was small making estimates of the incidence of adverse effects in this group unreliable (see Supplementary Table 6). Maternal adverse events are in Supplementary Table 7.

Discussion

This study provides an estimate of the real-world efficacy of continued maternal cART. The government of Malawi reviewed their experience of the efficacy of Option B+; but HIV status was documented at 12 months or beyond on only 54% of infants leading to an inability to make a reliable estimate of efficacy [23]. Furthermore, a recent review did not find any reports which were able to assess late MTCT through the end of 12 months of breastfeeding using option B or B+ [24]. We believe that our study is the first to report rates of MTCT at 18 months of age.

Predictive models to estimate the efficacy of different national programmes to lead to population wide HIV MTCT rates < 5% have suggested that very high population coverage with maternal ARVs will be necessary [25,26]. Our data showing 4.1% MTCT using continued maternal cART within the context of a clinical study suggest that even at 90% enrolment in ARV programmes nationally, achieving a population wide MTCT rate of less than 5% may be challenging [25]. Furthermore, loss to follow-up antepartum (15%) and postpartum (13%) would have led to a significant number of women discontinuing cART. This occurred despite using adherence counselling, food and transportation stipends and text messaging reminders to enhance follow-up. Therefore, the true intent-to-treat transmission rate would likely be

<table>
<thead>
<tr>
<th>Time interval (month)*</th>
<th>Number of deaths</th>
<th>Incidence rate (per 100 person-week)</th>
<th>Incidence rate ratio</th>
<th>95% CI of IRR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0–6)</td>
<td>12</td>
<td>0.2346</td>
<td>1.0000</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(6–12)</td>
<td>4</td>
<td>0.0838</td>
<td>0.3570</td>
<td>(0.1148, 1.1102)</td>
<td>0.03952</td>
</tr>
<tr>
<td>(12–18)</td>
<td>2</td>
<td>0.0433</td>
<td>0.1847</td>
<td>(0.0412, 0.8284)</td>
<td>0.00160</td>
</tr>
<tr>
<td>(18–24)</td>
<td>2</td>
<td>0.0457</td>
<td>0.1947</td>
<td>(0.0434, 0.8735)</td>
<td>0.00228</td>
</tr>
</tbody>
</table>

Note: *Patients contributed the respective person-time corresponding to each time period provided they were known to be remaining at risk of death during that time period. Therefore, those who were lost to follow-up at any point did not contribute person-time for subsequent time periods. Mortality at each time interval is compared with that at the first time interval (0–6 months). Confidence intervals were determined considering a Poisson distribution for the number of deaths. NA, not applicable.

Figure 2. Type of feeding over time.

Note: definitions for feeding types [24].
higher than 5% even within this study cohort. Very high “real-world” rates of loss to follow-up in pregnant and breastfeeding women on cART have been found in other studies [27–29] and so we feel that our data reflect real-world efficacy. The 95% CI for the MTCT rate was 2.2–7.6%, and so further studies with larger cohorts will be required to confirm the rate of MTCT using continued maternal cART.

Six episodes of post-partum transmissions occurred and of these, all five for which data were available to determine timing occurred after six months of age. Adherence to maternal cART was poor or questionable in all five women in our cohort who transmitted after six months (Supplementary Table 2). The transmission of a K65R resistance mutation leading to potential failure of future cART in one infant is concerning. A meta-analysis of 51 studies found that only 53% of women had adequate adherence to ARVs in the postpartum period [31]. Our data suggest that measurement of maternal VL at six months was predictive of subsequent HIV transmission. This is consistent with data involving heterosexual transmission of HIV, which demonstrated the importance of viral load in risk of transmission [31]. Our data suggest that measurement of maternal VL at six months of age may help to identify infants at higher risk of late transmission. Patients displaying virologic failure, or patients who have missed dispensary visits, may benefit from further counselling regarding ARV adherence, both for the mother’s health and to prevent MTCT although this approach would require further study to confirm. Maternal adherence to treatment may have been improved by using a daily rather than a twice daily cART regimen [32]. It is notable, however, that twice daily ZDV/3TC is still a preferred option in both the US and British treatment guidelines for pregnant women [33,34].

Our data support the logical premise that poor adherence leading to an elevated maternal viral load appears to be the main factor responsible for post-partum transmission. We base this on the observation that not only elevated maternal viral load but also missed dispensary visits were associated with transmission. However, the hypothesis that there may be something inherently different about the EBF (0–6 months) vs. the CF and COB (6–18 months) periods that makes the latter particularly susceptible to MTCT in the setting of an elevated viral load is also possible. The trend towards a higher transmission rate/100 person-weeks during 6–18 months when compared to 6 weeks to 6 months (p = 0.167) is suggestive. In addition, when including transmitting and non-transmitting mothers, more mothers had a detectable VL ( > 50) at birth than at six months (p = 0.002), and mean Log_{10} viral load at six months was not different from that obtained at birth, which would lead one to expect that there would be equivalent or more transmissions in the 0 to 6 months vs. 6 to 18 months period if VL level was the sole factor responsible for MTCT, which was not seen in our results. The mechanism of this potential inherent difference between the EBF (0–6 months) and CF/COB (6–18 months) period remains speculative but may include ingestion of contaminated water, fluids and food, which may lead to gut mucosal injury and disruption of immune barriers which has been proposed for the increased MTCT seen in previous studies [35,36] that identified a higher transmission rate with mixed feeding compared with EBF. This hypothesis should be interpreted with caution, however, given our low event rate, our VL comparator convenience sample being limited to 96 non-transmitting mothers and that there was a trend but not a statistically significant difference in transmissions in the 0 to 6 months vs. 6 to 18 months period.

Our HIV-uninfected infant mortality rate was highest in the 0 to 6 months’ time period (12 deaths) vs. any other time period (8 deaths in 6–18 months time period). Worldwide, a child’s risk of dying is highest in the first 28 days of life [37]; our results were consistent with this, with 8 of 12 HIV-uninfected infant deaths in the 0–6 month time period occurring in the first 28 days of life (Supplementary Table 5). In addition, the well-known ‘survivor bias’ would also predict this drop in mortality as time advances. Comparison of mortality rates in a specific time period (i.e. 6–18 months) to

Table 3. Total infant adverse events (excluding HIV-infected infants)

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Time period</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–6 months</td>
<td>6–12 months</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>18 (0.36)</td>
<td>55 (1.16)</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>12 (0.24)</td>
<td>11 (0.23)</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>88 (1.75)</td>
<td>34 (0.72)</td>
</tr>
<tr>
<td>Rash</td>
<td>71 (1.42)</td>
<td>15 (0.32)</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>14 (0.28)</td>
<td>6 (0.13)</td>
</tr>
<tr>
<td>Malaria</td>
<td>6 (0.12)</td>
<td>2 (0.04)</td>
</tr>
<tr>
<td>Nonspecific infection</td>
<td>14 (0.28)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>0 (0.00)</td>
<td>2 (0.04)</td>
</tr>
</tbody>
</table>

Note: Rates are based on per 100 person-weeks. *Patients contributed the respective person-time corresponding to each time period. *p-Values were computed using a Poisson regression.
a historic time period (i.e. 0–6 months) is biased in the direction of making the later mortality rate appear lower even though it is being compared within the same population. This is because of the premise that the population that is remaining at the later time period is actually a substantively different population by virtue of the fact that infants who have survived >6 months have already been selected out as being healthier/more resistant to mortality. The fact that this survivor bias was not seen in previous studies is interpreted as a complication of the early COB in those studies [9,12]. By contrast, in our cohort, the total and HIV-uninfected infant mortality rates fell significantly after six months, which suggests a protective effect of on-going breastfeeding.

Our absolute rate of infant mortality in the first six months of life was higher than that seen in the recently published BAN study in Malawi [9]. This difference may reflect the reported higher under-5 mortality rate in Zambia when compared to Malawi, the aetiology of which is probably multifactorial [38]. This fact, however, should not change the within-populations comparison of under and over 28-week death rates seen, which showed a significant increase in mortality after 28 weeks in the Malawi population which utilized early COB (from 0.065 to 0.12/100 person-weeks) vs. a decrease in mortality after 28 weeks in our cohort (from 0.2183 to 0.0755/100 person-weeks; p = 0.007). Overall, this would suggest that the difference in infant-feeding practices between the two studies is a very plausible explanation for the demonstrated reversal in pattern of the change in mortality at six months. Nevertheless, comparisons in outcomes between different cohorts need caution in their interpretation.

Our infant FTT was significantly higher during EBF when compared to later time periods (Table 3). We believe that this is also likely due to a survivor bias, and this also impacts early death rates as about 45% of all child deaths are linked to malnutrition [37]. After infants are developmentally advanced enough to accept significant amounts of semi-solid/solid foods (typically after six months), the available options to provide safe, high-caloric and nutrient-rich foods expand significantly.

Conclusions

Continued maternal cART can achieve the global UNAIDS target of <5% MTCT for eradication of paediatric HIV within the context of a clinical study but loss to follow-up and poor adherence to therapy may limit the benefit. Continued breastfeeding may prevent the rise in infant mortality at six months seen with early COB. These data will help to inform policy decisions regarding implementation of the new WHO guidelines.

Authors’ affiliations

1Department of Paediatrics and Child Health, University of Zambia, Lusaka, Zambia; 2Department of Pediatrics, Western University, London, Canada; 3Department of Public Health, University of Zambia, Lusaka, Zambia; 4Department of Epidemiology and Biostatistics, McGill University, Montreal, Canada; 5ISSS Medical Research, Montreal, Canada; 6Department of Medicine, University of Toronto, Toronto, Canada; 7Department of Obstetrics and Gynecology, University of Zambia, Lusaka, Zambia; 8Department of Anesthesiology, University of Zambia, Lusaka, Zambia; 9Department of Paediatrics, Arthur Davison Children’s Hospital, Ndola, Zambia; 10Department of Nursing, Chelstone Clinic, Lusaka, Zambia; 11Public Health Agency of Canada, Ottawa, Canada; 12Harvard Medical School, Cambridge, Mass; 13Grand Challenges, Ottawa, Canada; 14Division of Infectious Diseases, Western University, London, Canada

Competing interests

This study was partially funded by unrestricted grants from Abbvie and ViV Healthcare. None of the study funders had any role in study design, conduct of the study, study analysis or manuscript preparation.

Authors’ contributions

All authors have read and approved the final version.

Acknowledgements

We thank B. Picov for his strong support of this study. We also thank N. Ackad, M. Martinez, K. Vermeulen, A. Kopinska, M. Chipili, K. Semaru, R. Mahone, M. Nsowa, R.K. Silverman, P. Godfrey and N. Mbewe for their contributions. Finally, thank you to the women, children and staff of the Chelstone Clinic.

Funding

This study was supported by The Rotary Foundation, The Stephen Lewis Foundation, The Mount Sinai Hospital Foundation, ViV Healthcare and AbbVie. The funding agencies had no role in the design, analysis and conduct of the study or writing of the manuscript.

References


